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aerosol; aerosol generation; aerosol mass concentration; aerosol particle size; Cristobalite; evaporation-condensation generator; RA III

(cont'd on reverse)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

An inhalation exposure generation and monitoring system was develop and tested for exposing laboratory rats to: (1) solid particulate aerosols, (2) solid-liquid aerosol mixtures, (3) Cristobalite aerosol (postive control) and (4) filtered air (normal control). The test facility consisted of two laboratories: one with five 1 m^3 inhalation chambers used for exposures to test aerosols, and the second with two chambers for exposure of control rats to filtered air. The aerosol exposure chambers were interfaced with two types of aerosol genertors provided by the Government: one for the solid particulate aerosols and the second for the Petroleum Based Liquid (PBL) aerosols. Exposure chamber atmospheres were tested with solid particulate aerosols, with liquid aerosols and with solid-liquid aerosol mixtures. Aerosol mass concentrations were monitored continuously with optical sensors and periodically by gravimetric filter collection. (cont'd on reverse)

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18. SUBJECT TERMS (cont'd)

inhalation exposure
light scattering sensor
obscurant
particle dispersion
petroleum based liquid
pneumatic dispersion generator
smoke
solid-liquid aerosol mixture
spatial homogeneity
statistical evaluation
temporal homogeneity

19. ABSTRACT (cont'd)

Particle size distribution was measured by a Quartz Crystal Microbalance-based cascade impactor. Selected filter samples were analyzed by HPLC to show that neither the aerosolf generation process, nor the mixing of solid particulate and PBL aerosols produced any degradation of the PBL. Spatial and temporal homogeneity of aerosol concentration and particle size within the chambers were established through a procedure of simultaneous sampling. Statistical analysis of the data demonstrated that the total variation in aerosol concentrations was within the ±20% limits set for the study at all concentration levels tested. Although particle size showed a total variation exceeding the 20% limit, the individual variances attributable to the spatial and temporal components were always less than 20%, and particle size was always within the inhalable size range. On the basis of these results it was concluded that the inhalation chamber atmospheres showed spatial and temporal homogeneity for all aerosols tested in this study.

FOREWORD

This report, IITRI No. L06234 Phase I report, describes studies conducted by the Life Sciences Research Department of IIT Research Institute for the U. S. Army Medical Research and Development Command during the period of January 15, 1989 through February 1, 1990. The studies were carried out under Contract No. DAMD17-89-C-9043. Major David Smart was the Contracting Officer's Representative. Catherine Aranyi, Principal Investigator, was in charge of the overall conduct and coordination of the studies. Narayanan Rajendran, Co-Investigator, and Stanley Vana, Principal Professional Associate, were responsible for the design and operation of the test atmosphere generation and monitoring system and for the conduct of the aerosol homogeneity studies. Robert Gibbons, Consultant Biostatistician, contributed to the sampling design and conducted the statistical analysis of the aerosol homogeneity studies. Chemical analysis of selected components of filter-collected chamber atmosphere samples was the responsibility of Alan Snelson and Kevin Taylor. Robert Lange and Anthony Guy also participated in the conduct of the studies.

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NA For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institute of Health.

Catherine Aranyi 2/1/90

Catherine Aranyi PI Date

GOOD LABORATORY PRACTICES

The studies for this contract will be conducted in compliance with the Good Laboratory Practice (GLP) regulations as published in the Federal Register, Vol. 54 (158), 17 August 1989, pages 34043-34050 (and all subsequent amendments). This Phase I report summarizes the test atmosphere development studies and therefore the records were not submitted for quality assurance audit, but were subjected to rigorous internal quality control (for details see Section 5.4). All raw data generated during the course of the Phase I test atmosphere homogeneity studies will be retained in the IITRI Life Sciences archives and will be available for inspection.

Marilyn Marlow
Quality Assurance Auditor

EXECUTIVE SUMMARY

This study is sponsored by the U.S. Army Medical Research and Development Command to investigate the potential inhalation hazard of various materials employed as obscurants to which military personnel may be exposed during field operations. The overall objective is to evaluate the toxicity of aerosols and mixtures of aerosols by exposing laboratory rats in inhalation chambers under simulated field conditions. This program requires evaluation of the potential inhalation hazards of aerosols of a solid particulate material and mixtures of this solid particulate aerosol with a petroleum-based liquid aerosol. The subjects of the current Phase I report are the test atmosphere development and the chamber atmosphere homogeneity studies.

The facilities dedicated to these studies include two inhalation exposure laboratories provided with conditioned air supply and chamber air exhaust systems; inhalation exposure chambers with air flow and pressure controls; and aerosol generators provided by the Government. There are seven $1m^3$ inhalation chambers: four are used for exposure to aerosols of the test materials, and one is used for the positive control aerosol. Two chambers in a separate room are used to expose the control animals to filtered air. Each of the five exposure chambers is interfaced with two aerosol generators provided by the Government; one for the solid particulate aerosol and the second for the petroleum-based liquid (PBL) smoke. A two-stage filtration system, consisting of a bag prefilter and a HEPA filter, is used to exhaust the chamber atmospheres.

Aerosols of the solid test material are generated by pneumatic re-dispersion of the bulk solid using a jet mill and a screw feeder. The solids delivered by the screw feeder at a constant rate are drawn into the mill and are accelerated to high velocities with the use of compressed air jets. The particles are swept into turbulent motion and pulverize each other. Subsequently, they enter a size classifier and, if small enough, exit the system as aerosol. Large particles stay in the mill until their size is reduced to a level small enough to pass through the classifier. In order to improve the temporal stability of the solid aerosol generation system at concentrations below 100 mg/m^3, a slip stream dilution system was employed.

Aerosols of the PBL are generated by injecting this test material onto a Vycor glass heating element in an inert nitrogen atmosphere. The temperature of the heater is monitored by a thermocouple and controlled by a dual set point temperature controller. The PBL flash evaporates and condenses quickly to form smoke when mixed with dilution air. The solid particulate aerosol and the PBL smoke are dynamically mixed in a "Y" section before entering the chamber. HPLC analyses of the PBL aerosol collected on the filters revealed that the PBL did not degrade either during the generation process or when mixed with solid particulate aerosol.

The solids aerosol generator was tested to establish chamber aerosol concentrations within the range of 10 to 200 mg/m^3 (specific levels of 10, 60, 100, and 200 mg/m^3) and the PBL liquid aerosol generator in the concentration range of 500 to 1000 mg/m^3 (specific levels of 500 and 1000 mg/m^3). Both

generation systems functioned without any problem and the aerosol output remained stable at these concentration levels. We believe that the performance of the generators will be similar at any other concentration levels within the tested range. However, some readjustment of the generator settings will be needed for other aerosol concentrations potentially to be used in the upcoming exposure studies.

Three real time optical aerosol sensors were evaluated for monitoring the solid as well as solid-liquid aerosol mixtures. The extreme stickiness of the solid-liquid aerosol mixtures combined with high electrostatic charge of the solid aerosol produced particle deposition onto the sensor's optics and was a major operational problem. Sensors required cleaning within an hour or two of operation. Hence, gravimetric filter collection (at pre-set periodic intervals) was chosen as the primary method for determining the chamber aerosol concentrations. The real time sensors were used to monitor relative concentration variations continuously and serve as a guide for generator adjustment.

For studies with solid particulate aerosol only, a commercially available sensor, the portable continuous aerosol monitor (PCAM) was used to monitor aerosol concentration without any major operational problem. However, over the course of a 4-hr testing period, small drifts were noticeable and daily cleaning of the sensor was necessary. For studies with solid-liquid aerosol mixtures, it was necessary to monitor the concentration at three locations: at the solid aerosol generator outlet, the liquid (PBL) aerosol generator outlet, and in the chamber itself. A backscattering photosensor, originally designed by Oak Ridge National Laboratory (ORNL), was modified in our laboratory to provide a protective air sheath, and was used at the solid generator outlet and in the chamber (we call it "in situ sensor"). The output from the liquid aerosol generator was monitored with the original ORNL photosensor without any modifications.

The particle size distribution of the solid, as well as solid-liquid aerosol mixtures was determined by a Quartz Crystal Microbalance (QCM)-based cascade impactor. The mass median aerodynamic diameter (MMAD) of the solid particulate aerosol was in the range of 1.5 to 2.0 μm and for the solid-liquid aerosol mixture the range was 0.3 to 0.4 μm (for ratio of solid to liquid in the aerosol mixtures, see below). The particle size distribution of the positive control material (Cristobalite), measured with a Mercer Cascade Impactor, was approximately 3.0 μm MMAD.

Spatial and temporal homogeneity of the chamber test atmospheres was established through a procedure of simultaneous sampling from several locations within the chambers. Two homogeneity studies were conducted: the first for the solid particulate aerosols, and the second for the solid-liquid aerosol mixtures. The positive control material was tested as part of the solid particulate homogeneity studies. The test concentrations employed to establish the homogeneity of the chamber atmospheres were:

Solid particulate aerosols (mg/m^3) : 10, 60, 100, 200
Solid/liquid aerosol mixtures (mg/m^3) : 200/500, 200/1000, 0/1000
Positive control aerosol (mg/m^3) : 200

Aerosol mass concentration and particle size were measured at each concentration as a function of location and time. The homogeneity data collected were statistically analyzed to establish the total and individual components of variance, such as effects due to time, shelf, chamber, etc. (A comprehensive report on the statistical analyses prepared by R. Gibbons, Biostatistical Consultant to this project, is attached in toto in Appendix C).

Results of the statistical analyses revealed that the total variation of the aerosol concentration was within the required 20% limits for all the concentration levels tested. For spatial homogeneity, the variation attributable to shelf was always less than 10% for both the solid and solid-liquid aerosol mixtures at all the target levels. For temporal homogeneity, the variation dye to time was also less than 10% at all test runs except once (at the 10 mg/m^3 level of the solid aerosol only study). For the inter-chamber comparison, the aerosol concentration variations between exposure chambers were below 5% at all concentration levels.

For aerosol particle size, the data showed the total variations to exceed 20% most of the time, however, mean particle sizes were always within the inhalable range. In addition, the variations attributable either to shelf or time were always less than 20%, indicating that these variations were not due to an inhomogeneity with respect to time or location. Moreover, absolute changes in the particle sizes were quite small and therefore are expected to have no significance from the biological point of view.

Based on these studies, it could be concluded that adequate levels of spatial and temporal homogeneity were attained for the aerosol concentration and particle sizes in all the chambers and at all the target concentration levels tested.

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1. INTRODUCTION

This study is sponsored by the U.S. Army Medical Research and Development Command in its ongoing effort to investigate the potential inhalation hazard of various materials employed as obscurants.

During field operations, military personnel may be exposed to high concentrations of airborne materials for short daily durations, repeated over irregular periods for a number of weeks. Because the airborne materials may be present as a single material and/or as mixtures of materials, the objective of this program is to evaluate the toxicity of aerosols of these materials and their mixtures by exposing laboratory rats in inhalation chambers under simulated field conditions.

More specifically, the materials to be tested include a liquid-phase and a solid-phase test article. Thus the program requires the generation of test atmospheres that consist of liquid and/or particulate aerosols in the respirable size range. Laboratory studies have been conducted on the inhalation toxicity of a liquid petroleum-based aerosol, but information is required on the potential hazards of a solid particle material and mixtures of the liquid and solid particle aerosols.

The study consists of four phases:

Phase I: *Test atmosphere development and chamber* homogeneity studies;

Phase II: Four-week inhalation exposure study with aerosols of the solid particulate test material;

Phase III: Four-week inhalation exposure study with solid-liquid aerosols mixtures; and

Phase IV: Thirteen-week inhalation exposure study with solid-liquid aerosol mixtures.

This Phase I report describes the test atmosphere development studies including methods and procedures employed for aerosol generation and monitoring and the determination of aerosol homogeneity in the chamber atmospheres.

2. INHALATION EXPOSURE FACILITIES

The facilities dedicated to these studies (a floor plan is shown in Figure 2-1) include two inhalation exposure laboratories (Laboratories I and II) provided with conditioned air supply and chamber air exhaust systems; inhalation exposure chambers with air flow and pressure controls; and aerosol generators provided by the Government. There are seven Rochester-type, one-cubic-meter inhalation chambers, five of which are located in Laboratory I and are used for exposure to aerosols of the test materials. Two chambers are in a separate room (Laboratory II) for exposure of the control animals to filtered air and to prevent contamination and contact of these control animals with the test materials. Both laboratories share the same supply air but are connected to separate exhaust systems. In addition, the facilities include three animal rooms equipped with an automatic self-flushing watering system which services the cage racks. (Further details on animal rooms will be provided in the next Phase Reports under "Animal Care".) A room, located adjacent to the facilities (not shown in the floor plan) is used as a "Service Laboratory".

2.1 SUPPLY AIR

Figure 2-2 shows a schematic diagram of the conditioned air supply system for Laboratories I and II. Before entering the system, supply air passes through six 30 cm x 30 cm x 2.5 cm prefilters (40% efficiency for 0.9 μm particles), six 30 cm x 30 cm x 1.3 cm charcoal filters (grade 4 x 10, Type PBL), and is preconditioned with an 8.75 ton water-cooled air conditioning unit. Temperature and humidity are adjusted to maintain conditions of 24 to 27°C and 40 to 60% relative humidity (RH). An electric duct heater with an automatic control system maintains the required temperature range. Two steam humidifiers, one located at the air conditioning unit outlet and the other in the air inlet duct to the laboratories, supply the humidity which is controlled with a high-limit, 85%, pneumatic modulating controller. An automatic air handling control panel for regulating cooling, heating, and humidity is located in Laboratory I.

Laboratory I, used for the aerosol exposures, is maintained at a negative pressure relative to the access corridor and the adjoining Laboratory II which contains the chambers for exposure of the control rats to filtered air.

The single-pass conditioned air is introduced into the rooms at the rate of 18 to 20 changes per hour. The conditioned room air is introduced into the chambers through individual inlet filter assemblies consisting of a fiberglass coarse filter and a HEPA filter.

2.2 EXHAUST AIR

The exhaust from the aerosol exposure chambers is filtered with a two-stage filtration system and exhausted through a 20 cm diameter spiral, galvanized iron duct connected to the chambers (Figure 2-3). The combined exhaust from the five chambers is moved by a pressure blower (2 hp, 3500 rpm electric motor) capable of providing >500 l/min. air flow to each of the experimental chambers against 75 cm of water pressure and is exhausted above

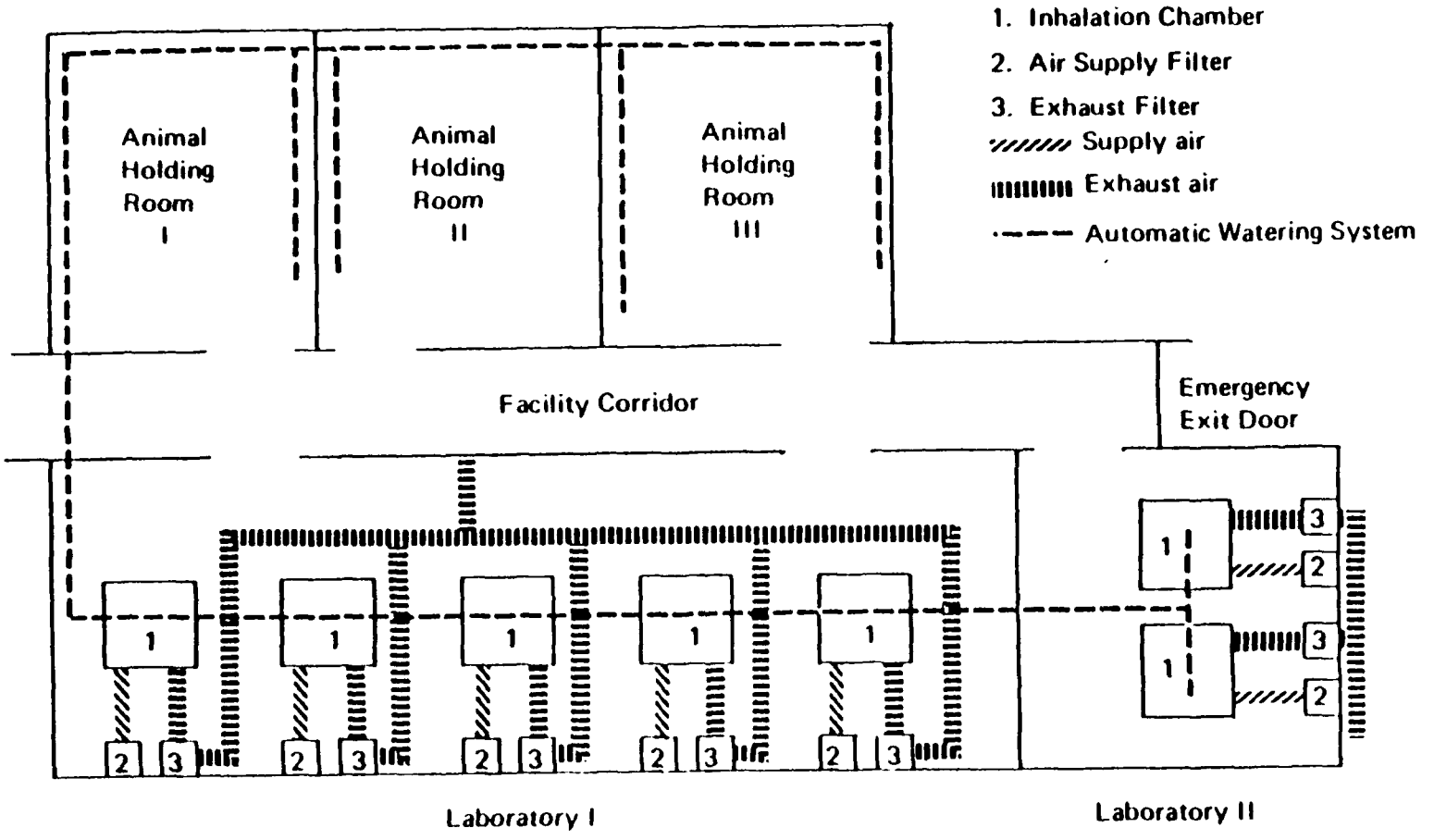


Figure 2-1. Floor plan of inhalation exposure facility.

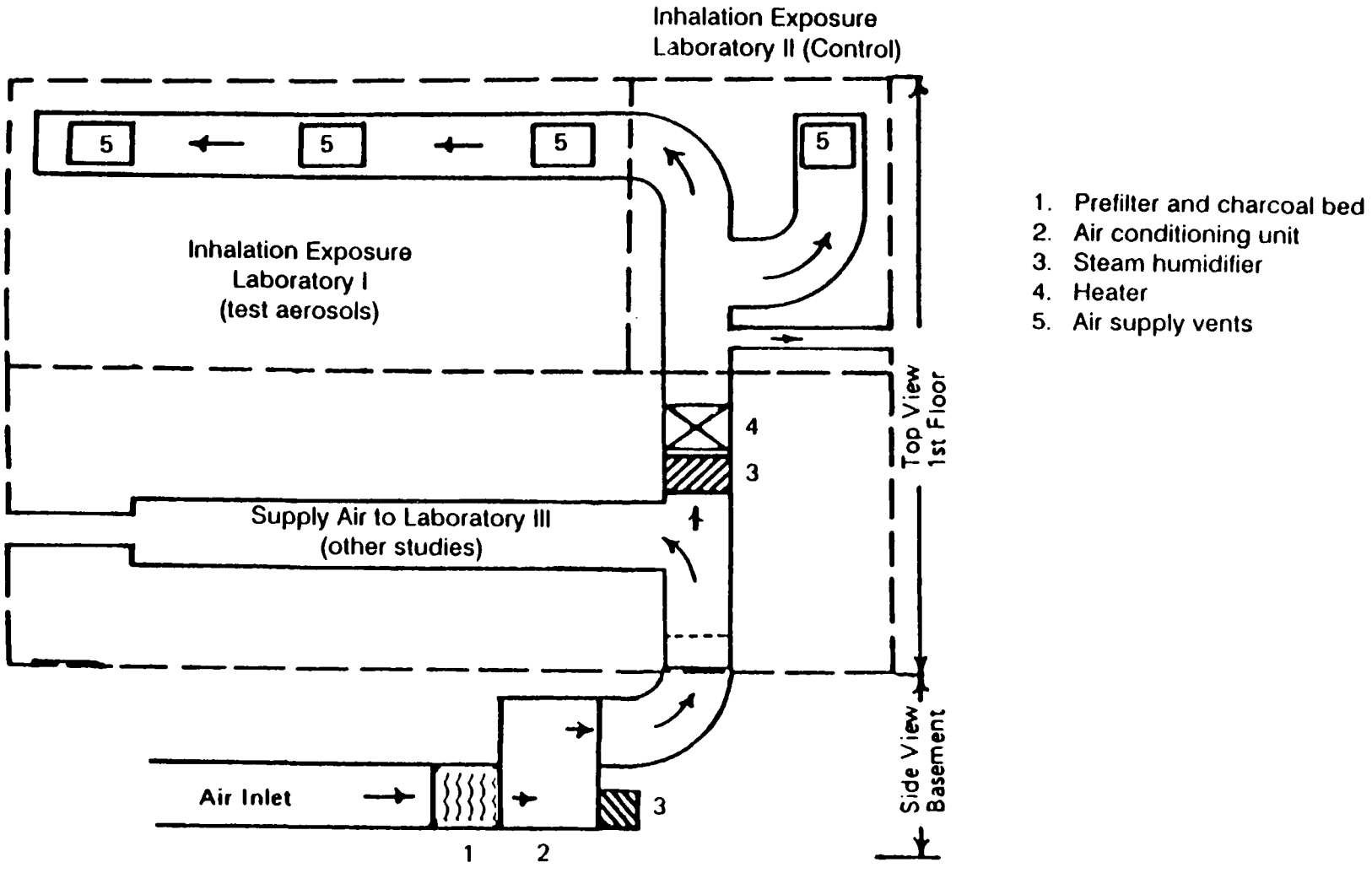
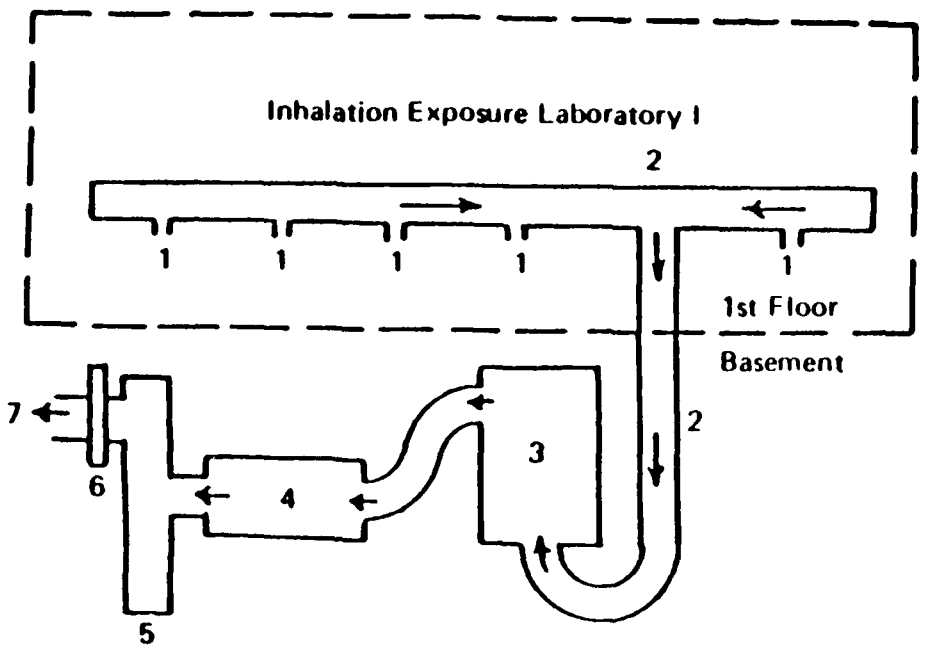


Figure 2-2. Schematic diagram of conditioned air supply system.



1. Filtered chamber exhaust
2. Exhaust ducting
3. Exhaust plenum
4. Silencer
5. Blower
6. Damper valve
7. Exhaust to outside

Figure 2-3. Schematic diagram of inhalation exposure chamber exhaust system (side view).

the roof outside the building. The individual chamber air flow is controlled and monitored with a valve located in the filtered exhaust of each chamber.

A wafer-type damper valve provides air flow control on the blower exhaust. A silencer filled with high density acoustical absorption material is installed between the blower and the plenum. The air moving equipment is remotely located to minimize noise in the exposure laboratory. The blower is connected to a switch over power supply that provides emergency power to the building. In case of blower failure an alarm system installed in the exhaust air provides warning to laboratory personnel to shut down the system.

The exhaust system for the control chambers is independent of the system for the experimental test chambers to avoid potential contamination with the test aerosols. Both exhaust systems are operated continuously except during chamber cleaning or maintenance.

2.3 AEROSOL EXHAUST FILTERS

Each of the exposure chambers is outfitted with its own filtration system consisting of a bag-type prefilter followed by a high efficiency filter. The prefilter is a Cambridge Model 3Z95 fiberglass filter which consists of five (5) bags or envelopes mounted in a parallel configuration. Overall, the filter is 30 cm x 60 cm x 73 cm in size, with the opening of the five bags on the 30 cm x 60 cm side. This configuration yields a filtering surface area of 48 square feet, has a rating of 93 $\frac{1}{2}$ 97% efficiency against atmospheric dust, and a holding capacity of 1 gram/cm^2. This is equivalent to 1.44 kg (3.2 pounds). The filter is mounted in an epoxy-coated plywood housing. The backup filter is also from Cambridge Filter Co. (Model 242412, Silver Seal). A Magnehelic pressure gauge is used to monitor pressure drop across these filters and determine filter loading.

This filtering system permits operation of the generating system for extended periods of time without shutdown. As the filters load and the pressure drop increases, adjustments must be made to maintain correct air flow. However, the loading is slow enough that only minor adjustments are necessary over the course of several weeks.

The data from the aerosol generator development study at Oak Ridge National Laboratory (ORNL) indicate that aerosol removal is a significant problem with the aerosol mixture (J. H. Moneyhun, T. M. Gayle, and R. A. Jenkins "A system for Generating Mixed Aerosols from a Petroleum-Based Liquid and a Solid", ORNL Draft Final Report). The aerosol mixture will completely seal the surface of the filtering material so the air flow is completely stopped. Experience has shown this to occur rapidly with some of the filtering systems tested (ORNL Study). As air flow is restricted, the danger is that the concentration of the aerosols increases and the petroleum-based liquid (PBL) could rapidly reach an explosive level.

In order to prevent such dangerous conditions, the air flow through the chamber is continuously monitored by an orifice meter. Also, the pressure drop across the filter systems is measured by a magnehelic gauge and is monitored by the laboratory personnel on an hourly basis. These two measurements indicate the degree of plugging and provide advanced indication of the need for a new filter.

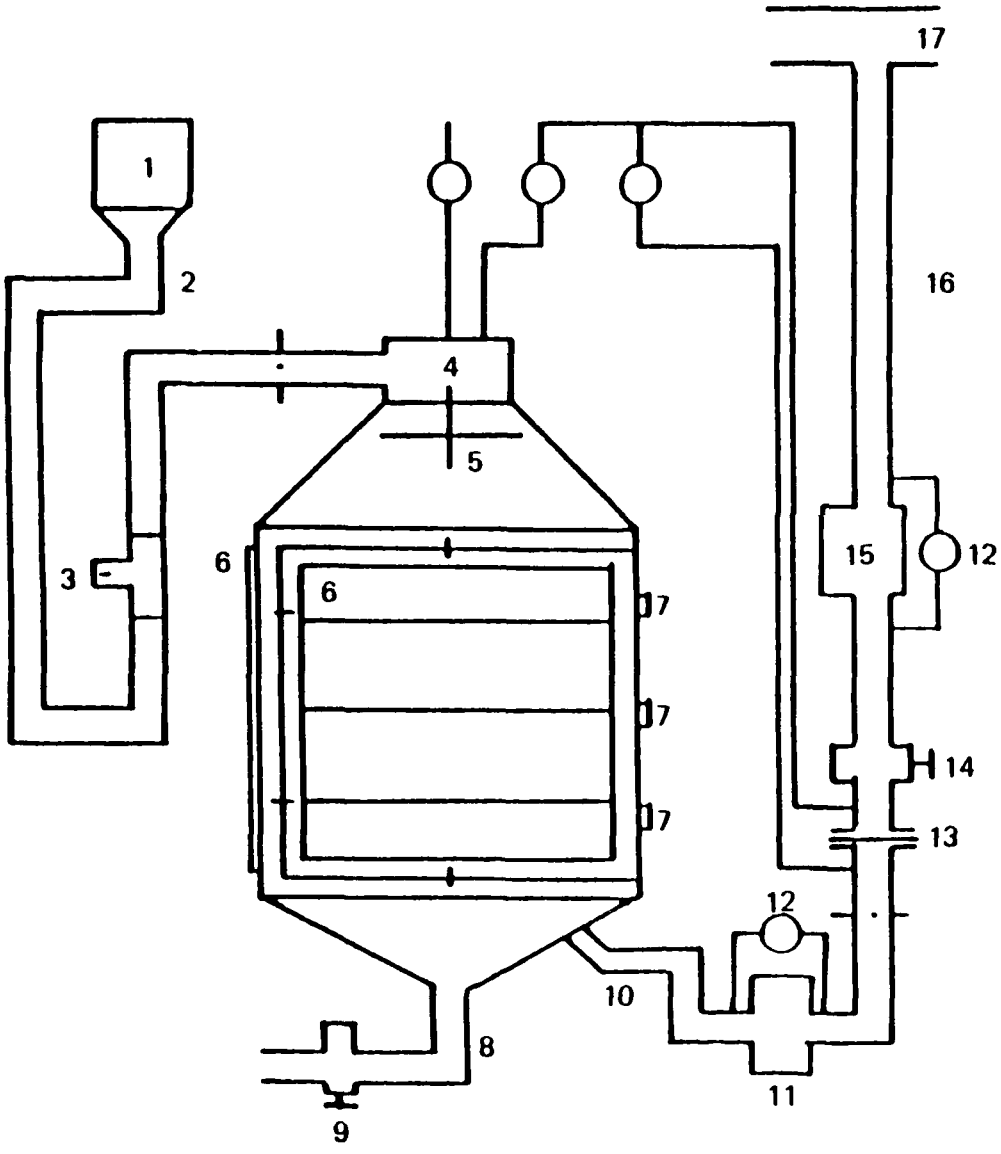
2.4 INHALATION EXPOSURE CHAMBERS

A total of seven (five for aerosols and two for filtered air) stainless steel inhalation exposure chambers are being used. Each is approximately one cubic meter in volume, which includes a central cubical (91cm x 91cm x 91 cm) and two pyramidal sections on the top and bottom, respectively. A door is located on the front of the chamber. Wire-reinforced glass windows are in the door and on one side wall of the chamber. Three sampling ports are located on the side wall opposite the window.

A schematic diagram of an exposure chamber is shown in Figure 2-4. Test material and dilution air are introduced tangentially into a mixing compartment. A cylindrical baffle plate, located just below the mixing compartment, aids in the uniform distribution of the test material in the main section of the chamber. Adjustable perforated shelves hold the individually-housed test animal exposure cages. The cages and chambers are designed to hold a maximum capacity 22 four-compartment rat cages, thus housing up to 88 rats. A gate valve at the bottom is used for washing and draining the chambers after each exposure period.

The chamber exhaust, located in the bottom section, passes through the wall of the chamber and into the primary and HEPA exhaust filters. An orifice meter monitors the air flow. The total air flow through the chamber is adjusted with a PVC valve located on the vacuum side of the orifice plate. The chamber air flow rate is calibrated with a mass flow meter and monitored by measuring the pressure differential across the orifice plate. A second gauge monitors the differential pressure between the chamber air inlet and exhaust to measure the total potential draw through the chamber. In addition, a differential pressure gauge continuously monitors the negative pressure in the chamber relative to the room air pressure. Individual chamber temperatures and percent relative humidity values are recorded three times each working day with an electronic thermohygrometer.

The air supply is capable of providing a per chamber flow rate of 0.5 equivalent volumes per minute (500 l/min) and meets the minimum flow rate requirement of 0.4 equivalent volumes/min necessary for the study.



1. Inlet air filter
2. Flexible inlet duct
3. Generator input (solid and liquid)
4. Mixing compartment
5. Deflection baffle
6. Reinforced glass windows
7. Sampling ports
8. Drain
9. Gate valve
10. Exhaust air line
11. Primary filter
12. Differential pressure gauges
13. Limiting orifice
14. Exhaust air control valve
15. HEPA filter
16. Flexible exhaust duct
17. Exhaust duct

Figure 2-4. Schematic diagram of inhalation exposure chamber.

3. TEST ATMOSPHERE GENERATION

Two types of aerosol generators are used to generate the aerosols and aerosol mixtures to be evaluated in these studies: one to disperse solid particulates (test material and positive control material) and another for the petroleum-based liquid (PBL) test material. Both types of generators were provided by the U.S. Army Medical Research and Development Laboratory through ORNL.

3.1 TEST MATERIALS

Three materials are used for this study. The two actual test articles are a petroleum-based liquid (PBL) and a solid powder. In addition, crystalline silica (Cristobalite) was included in the homogeneity tests because it will serve as a positive control during the Phase II studies.

The PBL and the solid test material were provided by the Government. Fifty-five (55) gallons of the PBL and 35 kg of the solid material were received from the Transportation Officer at Aberdeen Proving Ground, Maryland. Approximately 10 kg of Cristobalite was procured from CED Corporation, Cuyahga Falls, Ohio. All test articles are stored under ambient conditions until used in the aerosol generators. The solids test material was stored in the original containers, as received, until used (material is in a plastic bag inside a fiber drum). The PBL was stored in a metal drum, as received, and removed as required to fill the PBL generator reservoirs.

3.2 PETROLEUM-BASED LIQUID AEROSOL GENERATOR

The five generators were built by ORNL based on a generator developed there for generating an aerosol from diesel fuel (R. W. Holmberg, J. H. Moneyhun, T. M. Gayle, "Generating, Monitoring, and Controlling Petroleum Aerosols for Inhalation Chamber Studies". AD 134214 ORNL TM 8903). The generators employ an evaporation/condensation technique for generating the aerosol. A brief description of the generator is provided here. For additional details and design requirements, the reader is referred to the ORNL Report (J. H. Moneyhun, T. M. Gayle, and R. A. Jenkins "A System for Generating Mixed Aerosols from a Petroleum-Based Liquid and a Solid", ORNL Draft Final Report).

A schematic diagram of the PBL generator is shown as Figure 3-1. A one (1) kilowatt Vycor-encased immersion heater is mounted in a 2.5 cm (1-inch) diameter stainless steel tube. The temperature of the heater is monitored by a thermocouple and controlled at 1100°F by a Barber Colman Temperature Controller. When the temperature exceeds the preset temperature limits, the controller automatically cuts off the power to the heater and the oil pump. The fuel is pumped at a constant rate onto the surface of the Vycor-encased heater where it is flash evaporated. Nitrogen carrier gas (≈ 12 l/min.) sweeps the vapors out of the generator into a stream of dilution air where the vapors are cooled and condensed to form the aerosol. Nitrogen carrier gas is used to prevent a chemical reaction on the hot surface of the Vycor heater (using air as the carrier gas might generate an explosive mixture in the

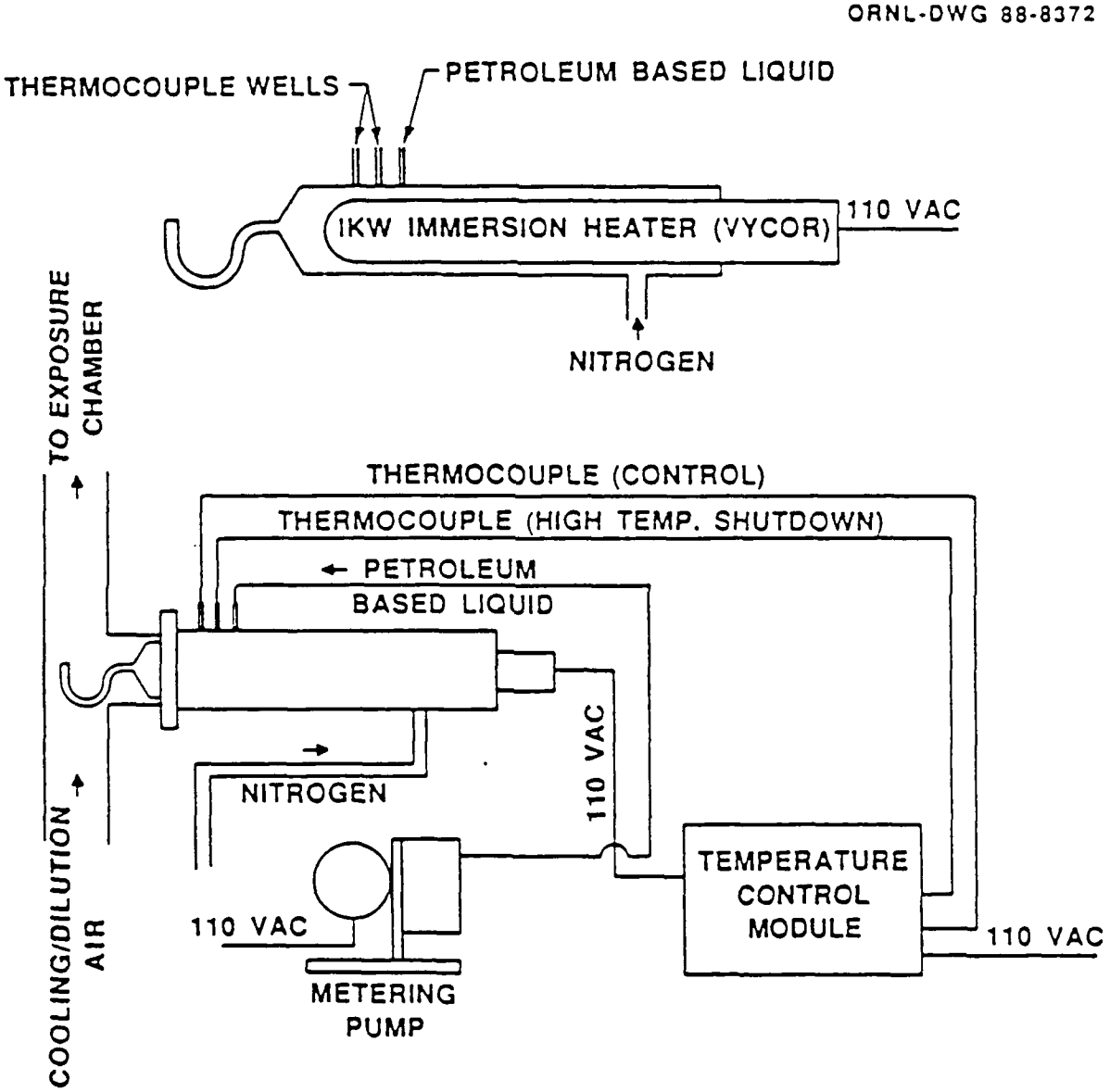


Figure 3-1. Petroleum based liquid aerosol generator.

generator tube). Because the exposure chamber operates at constant air flow rate (500 l/min), the aerosol concentration is regulated by varying the injection rate of the oil. Oil flow rates from 0.3 to 3 ml/min produce aerosol concentrations of 0.5 to 5 g/m^3. The generator is interfaced to the chamber air supply through a glass pipe tee (Figure 3-1). The hot gases from the heater and the dilution air mix turbulently to form a dense aerosol.

3.3 SOLID PARTICULATE AEROSOL GENERATOR

For these studies, a commercially-available system has been modified at ORNL to better disperse the candidate solid material (J. H. Moneyhun, T. M. Gayle, and R. A. Jenkins "A system for Generating Mixed Aerosols from a Petroleum-Based Liquid and a Solid", ORNL Draft Final Report). The system consists of a jet mill dispersing unit (Jet-O-Mizer Model 00, Fluid Energy Processing and Equipment Company, Hartfield, PA) fed by a screw feeder (Series 100 Accurate, Whitewater, WI). The rate of dispersion is controlled by the revolution rate of the feeder screw. Solids delivered by the screw feeder into the jet mill funnel are drawn into the mill by aspiration and are accelerated to high velocities by two air jets. The particles are swept into turbulent motion and pulverize each other. They are then swept into a classifier and, if small enough, are carried from the system into the delivery tube. Large particles are returned to the mill for further size reduction. Figure 3-2 is a schematic diagram of the solid dispersal unit. The jet mill operates with compressed air at 100 psi. Typically, air flow rate through the mill is approximately 100 l/min.

The screw feeder metering accuracy is improved by the stirrer that moves up and down over the feed screw. The stirrer is hinged at the front of the screw, made of 0.3 cm diameter stainless steel rod, and is vibrated by an air driven vibrator. The stirrer bar prevents bridging over the screw. A second vibrator attached to the delivery tube helps prevent peaking in the delivery tube. The delivery rates from the screw feeder were constant and varied only by a few percent (See calibration tables for screw feeder in Appendix A).

The solids generator is interfaced to the chamber air supply through a glass tee (Figure 3-2). The solid aerosol enters the tee at right angle and impinges on the wall. Larger particles and agglomerates impact on the wall and are captured. The fine particles are carried away to the exposure chamber by the dilution air (approximately 150 l/min.). The solid aerosol concentration in the chamber is regulated by varying the revolution rate of the screw. Typically, the aerosols generated with jet mills are highly charged electrically and it is customary to neutralize these particles before they enter the exposure chamber. However, generators used in this study have no provision to neutralize the electric charge. Our discussions with ORNL personnel (developers of the generators) revealed that the solid test material dispersed under field conditions also carries electric charge and therefore, the laboratory aerosol generators were also designed to simulate the field aerosol. The electric charge on the aerosol increases the deposition onto surfaces and therefore necessitates cleaning of the aerosol sensors in the chamber (refer to Section 4 for further details).

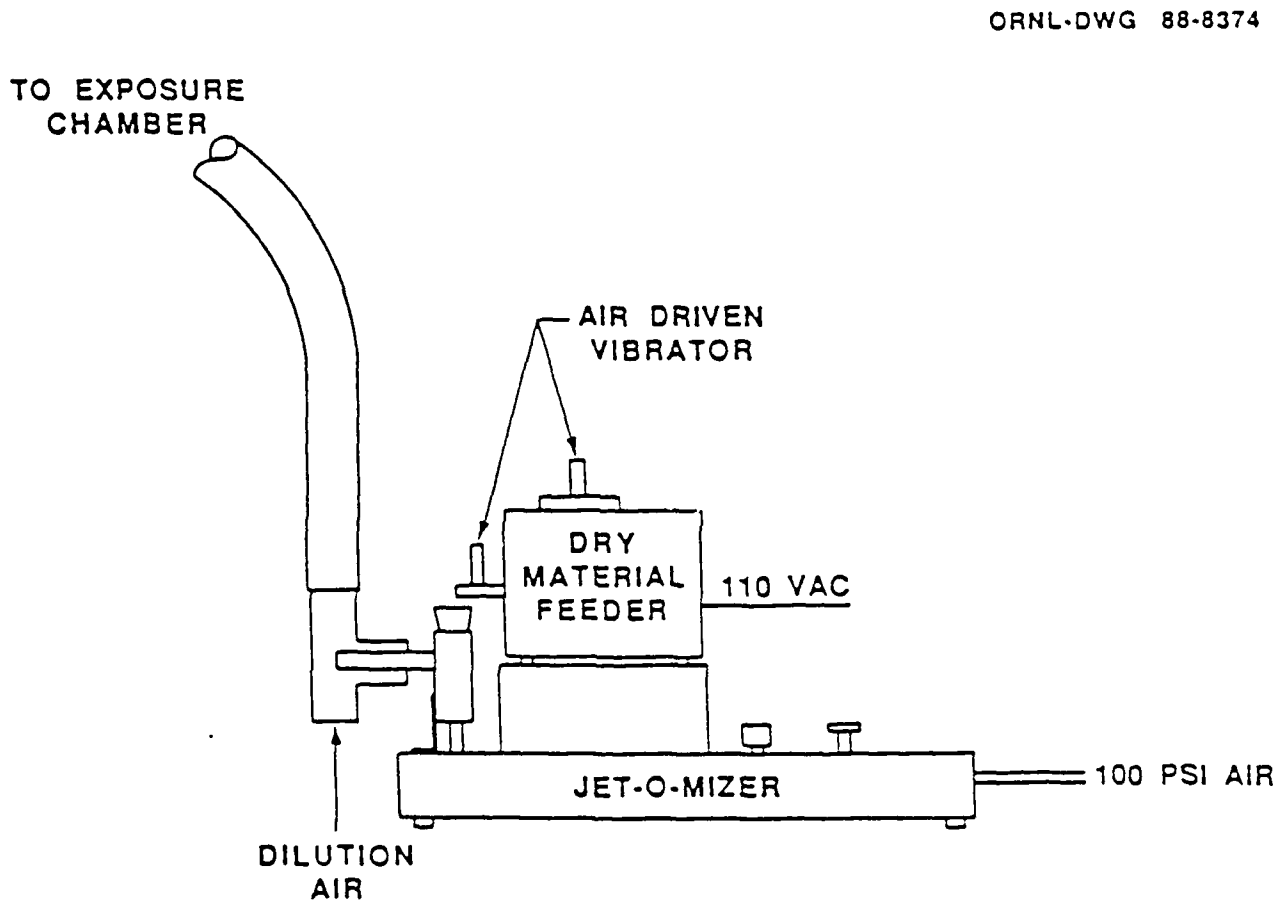


Figure 3-2. Solids dispersal system.

3.4 AEROSOL DILUTION SYSTEM

The solids aerosol generation systems operate consistently and reproducibly at high screw revolutions of the solids feeder. Based on the performance data, we established that these generators cannot produce test atmospheres in a reproducible manner at concentration levels below 100 mg/m^3. However, two of the aerosol concentration levels that were required for the homogeneity study (10, 60 mg/m^3) are below this stable operational level of the generator and hence modifications to the solid generation system were needed.

We employed a slipstream dilution system to solve this problem (Figure 3-3). With this dilution system, the solids feeder was operated at high enough feed levels that typically produce stable output; the required target aerosol concentration levels were achieved by controlling the aerosol flow rate to the chamber. The dilution system consists of a Venturi air mover (Vortec Corp., Cincinnati, OH), a HEPA filter and a control valve. The Venturi air mover is used to control the aerosol flow rate to the chamber. The air mover is powered by compressed air and is similar to a vacuum pump with no mechanical parts. The vacuum is controlled by adjusting the compressed air pressure to the air mover. The pressure difference across the chamber inlet and the venturi outlet is monitored by a pressure gauge (Figure 3-3) and is used as the basis for monitoring aerosol flow rate to the chamber.

3.5 AEROSOL EXPOSURE SYSTEM

Figure 3-4 is a schematic diagram of the aerosol exposure system showing the complete assembly of the various components. The exposure system consists of: supply air and carrier N_2, exposure chamber, solid particulate aerosol generator, liquid aerosol generator, chamber exhaust system, aerosol dilution system, and aerosol concentration monitors with associated photosensor data acquisition system. Each of the above components, except the aerosol monitors have been described earlier. Aerosol monitors are described in Section 4. For studies with mixed aerosols, the aerosol output from the liquid and solid generators are blended together in a "Y" section prior to entering into the exposure chamber. For studies testing solid particulate aerosols only, filtered air is pulled through the liquid aerosol generator side of the "Y". Also shown in the figure are the monitoring locations, three for the aerosol sensors and one for the gravimetric filter collection. Details on the aerosol monitoring approach and methods are given in Section 4.

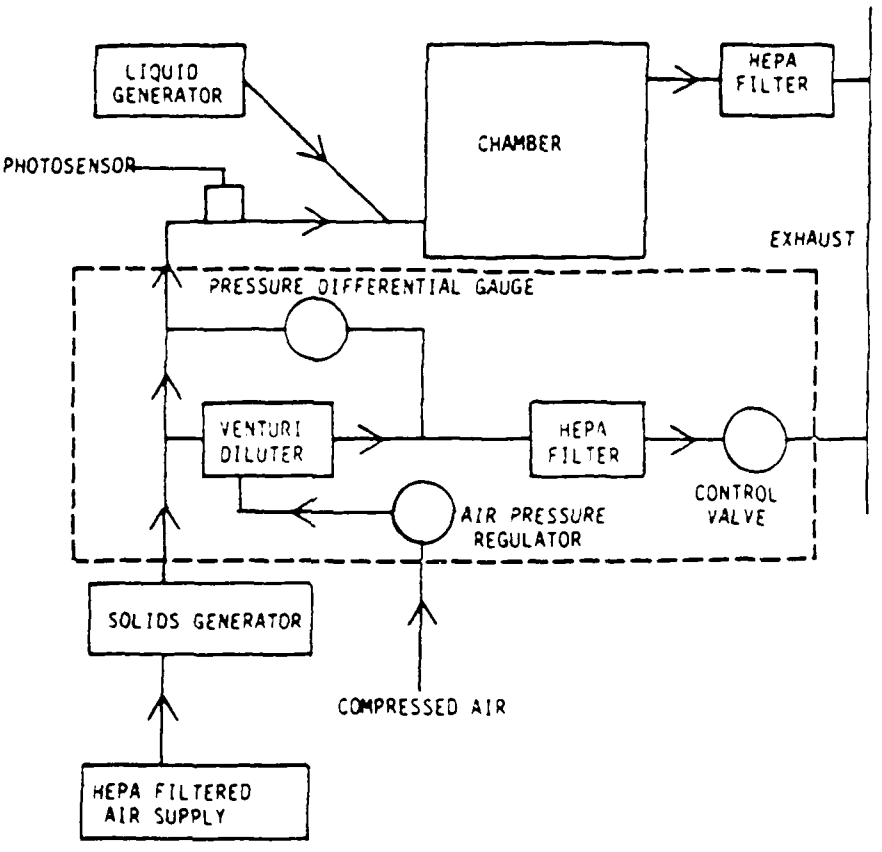


Figure 3-3. Solid particulate aerosol dilution system.

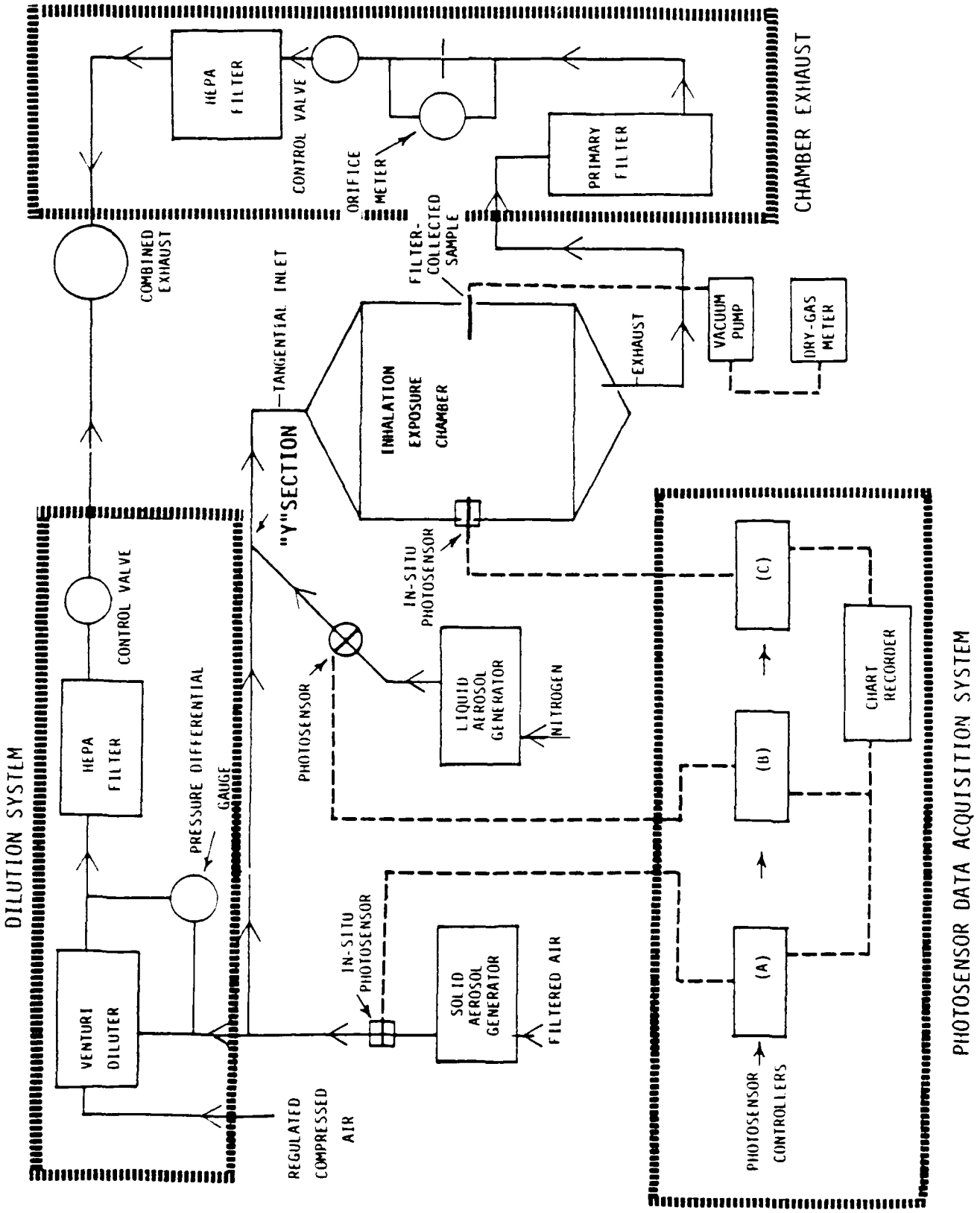


Figure 3-4. Schematic diagram of aerosol exposure system.

4. TEST ATMOSPHERE MONITORING

4.1 PRELIMINARY EVALUATION OF REAL TIME AEROSOL SENSORS

Because the ratio of liquid to solid aerosol concentrations can be as high as 10:1 (the typical target aerosol exposure concentrations are about 1000 mg/m^3 for the PBL and 100 to 200 mg/m^3 for the solid particles);, the concentrations of the liquid aerosol, the solid particles, and the mixture must be monitored individually. Hence a total of three aerosol sensors will be required for each chamber: one each at the solid and liquid aerosol generator outlets and one in the exposure chamber (see Figure 3-4 for locations). Three aerosol sensors were evaluated for potential use as real time monitors.

RAM Sensor: The first, a commercially-available real time aerosol monitor (RAM), MIE, Inc., Bedford, Massachusetts, utilizes a pulse light-emitting diode in combination with a silicon detector which senses the light scattered over a forward angle of 45° to 95° by the particles transversing the sensing volume. The sensor provides an analog output directly proportional to the aerosol concentration. The RAM operated satisfactorily at concentrations below 200 mg/m^3. However, when the instrument was outfitted with a diluter for concentrations higher than 200 mg/m^3, the response was not reproducible and as a result, no further evaluation of the RAM was done.

PCAM Sensor: The second sensor used to monitor chamber aerosol concentration of solid particulates is the Portable Continuous Aerosol Monitor (PCAM) real time aerosol sensor (PPM, Inc., Nashville, TN). The PCAM Model TX is a microprocessor-based electrooptical system which measures aerosol concentrations by the principal of near forward scattering of light emitting diode radiation. The unit has a control module that accepts and averages data over a preselected time period from five PCAM sensors, one used for each exposure chamber. The multiple sensors are connected to the central control/readout unit by individual cables. The control unit provides a real time digital readout of any one of the individual sensors. The control unit has the capability to average data over periods of 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 6 hours, and 8 hours, and can display concentration levels instantaneously. A printer provides a hard copy update of all sensor-averaged readouts. The sensors have self-contained sampling systems which operate at 1 l/min. flow rates. The concentration range of the sensors is 0-200 mg/m^3. The sensors have an internal self-calibration cycle that occurs once every hour. The calibration requires two minutes during which a zero value and a traceable factory calibration value are internally acquired. If either one of these values are outside the normal operating range, the system relays an error code to the output. The PCAM operated satisfactorily with only the solid particles. With the solid-liquid mixture aerosol, the particles could not be kept away from the optics of the sensor and as a result the sensor's calibration could not be maintained. Therefore, the PCAM sensor is used only for the solid particulate aerosol studies.

Backscattering Sensor: The third sensor, a light emitting diode (LED), is based on the backscattering principle and has been successfully employed in our laboratories to monitor the aerosol concentrations in several inhalation studies in the past, including the Red Phosphorous/Butyl Rubber (RP/BR) smoke study conducted for the USAMBRDL (Aranyi, C., "Research and Development of Inhalation Toxicologic Evaluation of Red Phosphorous/Butyl Rubber Combustion Products - Phase I", August 1983, A.D. A157686). The sensor's design was originally developed at ORNL (T. M. Gayle, C. C. Higgins, and Stokely, J. K., "Sensor for Detection of Tobacco Smoke in Inhalation Exposure Systems", 1979 ORNL-5424) and provided to us for use in the RP/BR studies. The backscatter probe consists of an LED and a sensor attached to a 1/4" diameter tube. The probe was evaluated in conjunction with several types of sampling and sheath air designs. Of the several designs evaluated, the two most promising types of sheath air systems with which we have had limited success are shown in Figures 4-1 and 4-2.

In the first design, the sensor measures the aerosol concentrations in the chamber in situ. A sheath of filtered compressed air flows over the sensor to prevent deposition of aerosols on the sensor (Figure 4-1). The in situ probe design operated satisfactorily with both the solid particles and the solid-liquid aerosol mixture. The probe could not be kept completely free of aerosol deposition and therefore periodic cleaning was necessary. For cleaning, the probe can be removed from the chamber without shutting the aerosol generators. Typically the sensors are returned to the chamber after cleaning within two minutes. Typical response traces from the in situ sensor for the solid aerosol and the solid-liquid mixture are shown in Figure 4-3 A, B, and C. The traces demonstrate that the probe is capable to monitor the concentration for over a two-hour period without any drift and sense the changes in chamber aerosol concentrations which are brought forth by varying the generator output.

In the second design, from hereon referred to as the external monitor, the concentration measurement is done on a sample removed dynamically by a vacuum pump from the chamber and the sensor itself is located externally to the chamber. An annular sheath of clean air prevents the deposition of particles on the probe (Figure 4-2). The external monitor design operated satisfactorily with both the solids and the solid-liquid mixture. But the sensor's response degraded in this design and lacked reproducibility. Therefore the external monitor design was not selected for use in the exposure studies.

In summary, the backscattering aerosol photosensor seems to work well if the particles can be kept away from the probe. The in situ design proved to be suitable for studies with both solid aerosol and the solid-liquid aerosol mixture, is much simpler to use, and does not require any sampling equipment. However, some routine cleaning of the photosensor is necessary. Therefore, we fabricated the in situ design sensors for monitoring the mass concentration of aerosol mixtures in the chamber and at the outlet of the solid generator. The liquid generator output was successfully monitored with the original ORNL designed backscattering photosensor itself without any modifications. For the solid only studies, either the PCAM or the in situ design sensor can be used to monitor the mass concentration of the solids in

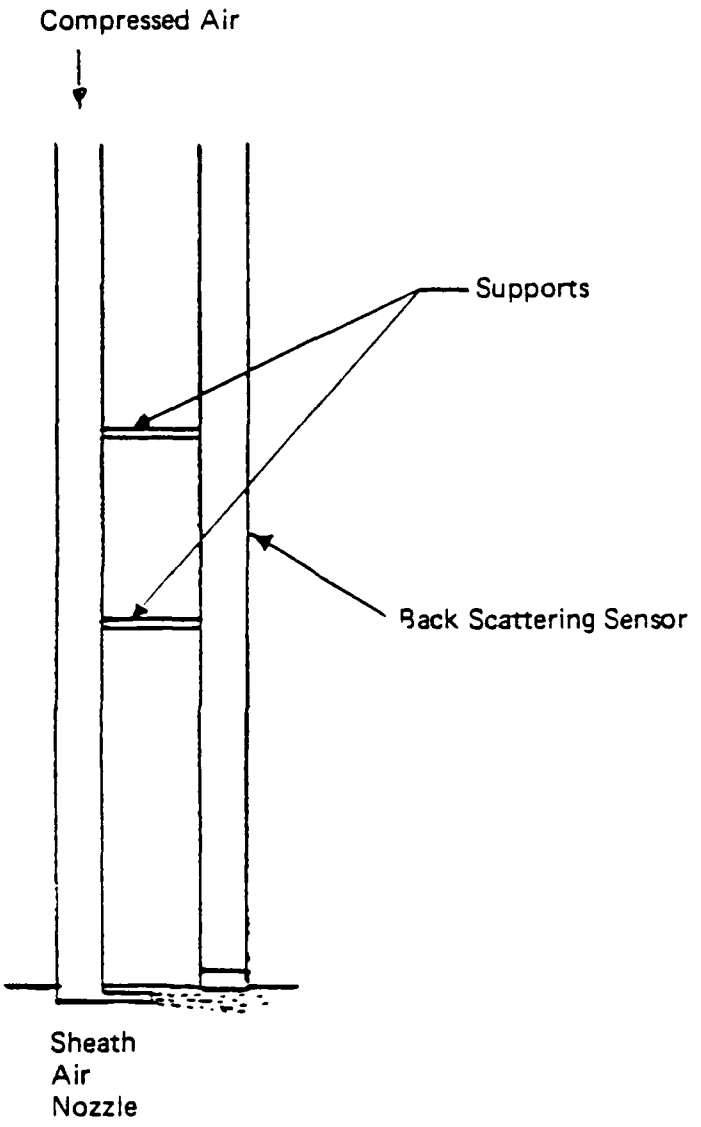


Figure 4-1. Backscattering photosensor: In-situ design.

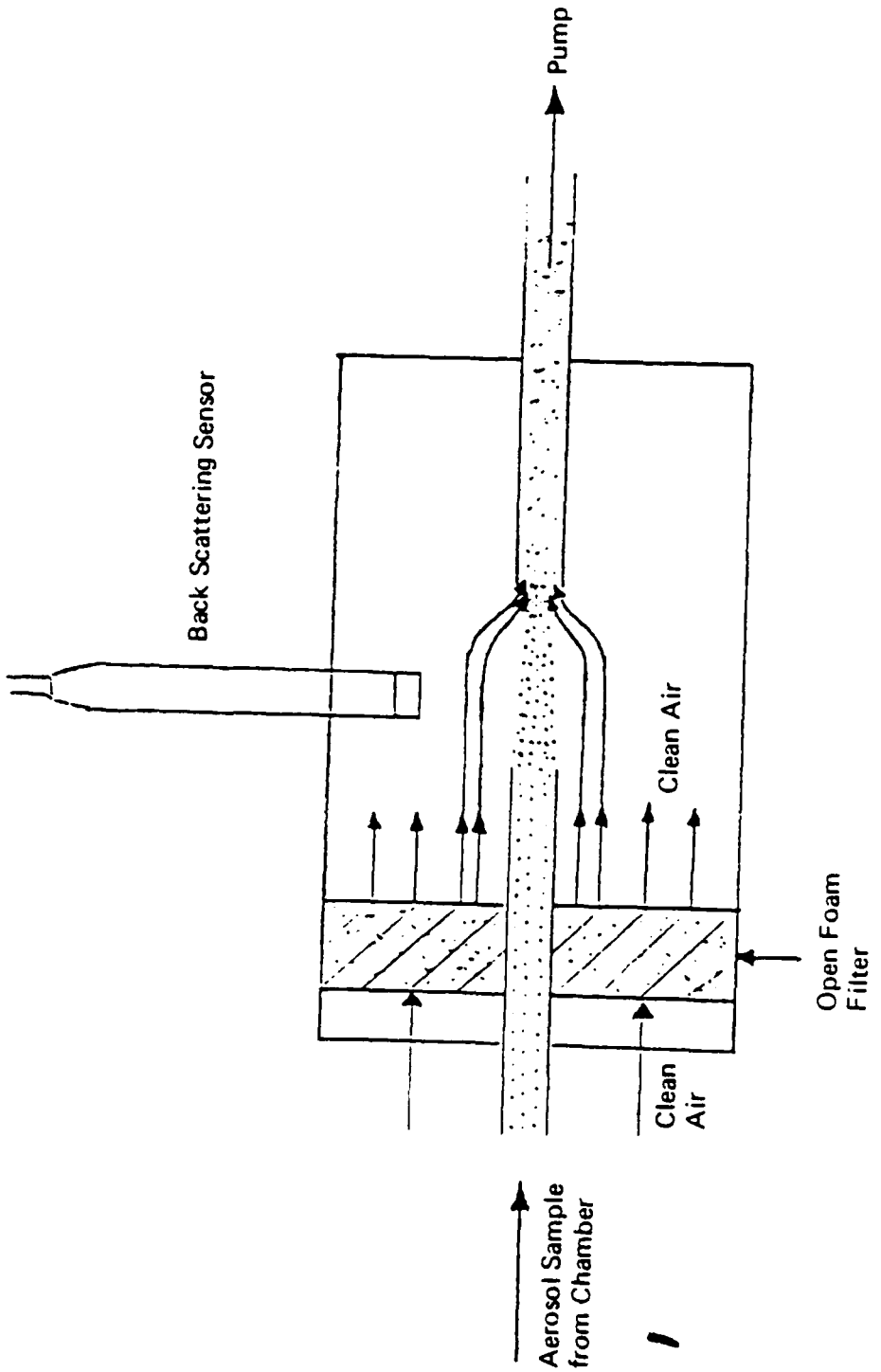
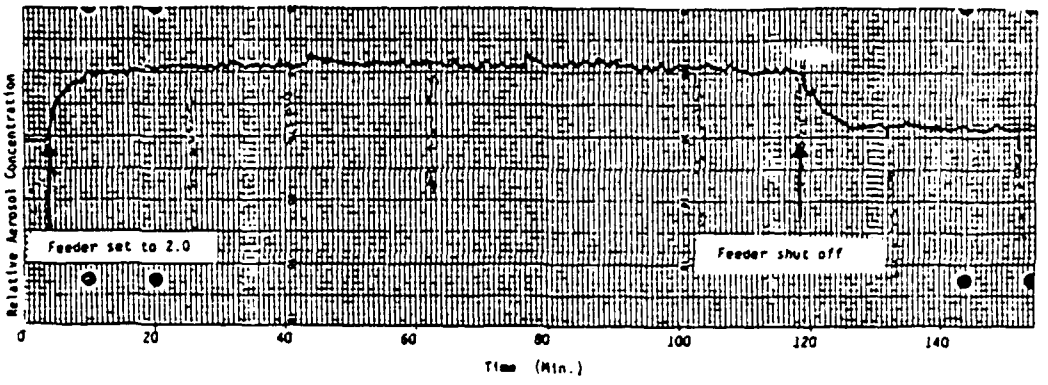
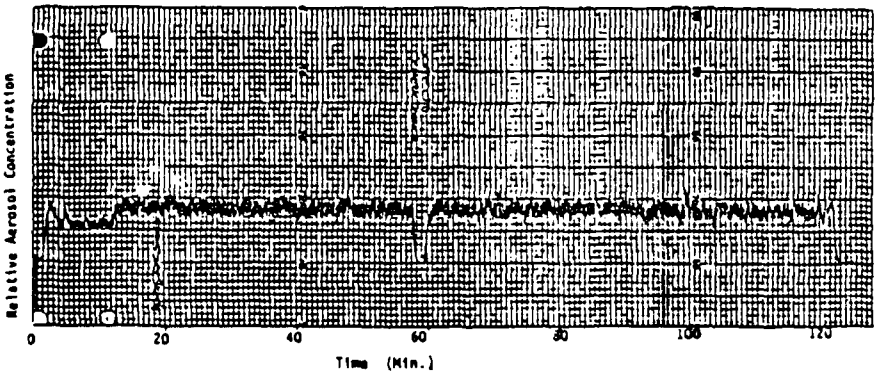


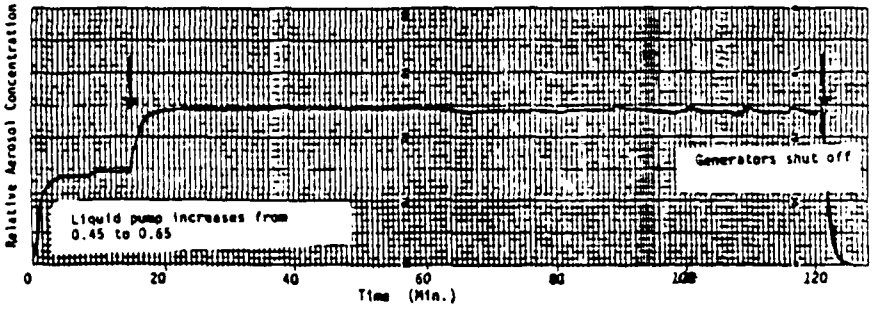
Figure 4-2. Backscattering photosensor: External design.



A. For solid particle in the exposure chamber.



B. At the outlet of the solid generator.



C. For solid-liquid mixture in the exposure chamber.

Figure 4-3. Response traces of In-situ design photo sensor.

the chamber. However, the PCAM sensor does not require the routine cleaning that is needed with the in situ sensor. Therefore, the PCAM sensor was selected for the studies using only the solid particulate aerosol.

In view of the difficulties associated with the real time monitoring sensors, the gravimetric method was chosen to be the primary method to monitor the aerosol concentration. The real time aerosol sensors were used as on-line guides to keep the aerosol concentrations at target levels. The aerosol particle size was monitored with QCM cascade impactors. Table 4-1 presents a summary of various sampling methods and proposed frequencies that were used to monitor the chambers during these studies.

4.2 AEROSOL MASS CONCENTRATION SAMPLING METHODS

4.2.1 Gravimetric Method

Aerosol mass concentration was monitored gravimetrically, approximately once for each hour of the testing period. Particles of the test aerosol were collected on pre-weighed 45-mm fiberglass filter disks placed in acrylic plastic filter holders. The filters have a 99.99 percent retention efficiency for dioctyl phthalate particles of 0.3 μm. Prior to use, the fiberglass filters were maintained for 24 hours in the conditioned atmosphere of the sampling environment to assure moisture equilibration by the filter pads. The aerosol samples were collected at constant flow rates, using diaphragm-type vacuum air pumps. The filters were weighed on an analytical balance. Dry gas meters connected to the backside of the pumps recorded the corresponding total volume of air sampled.

All filter samples were weighed within 30 minutes of removal from the sampling ports, transferred to plastic petri dishes, and entered into a permanent record. Selected filter samples of mixed aerosols were subsequently submitted for chemical analysis to determine the quantity of oil.

For aerosol homogeneity testing, the chamber doors were temporarily replaced with a specially-constructed plastic panel fitted to the front of the exposure chambers. A series of holes drilled into the plastic provided access for tubular stainless steel sampling probes, 98 cm long and 0.9 cm in diameter, to predetermined sampling locations inside the chamber. The filter assembly was connected to the end of each probe outside of the chamber. This design assured uniform sampling of the aerosol and also assured that the aerosol samples always traveled the same distance from the sampling point to the collecting filter (For detailed description of sampling locations, see Section 5).

4.2.2 Lightscattering Method

Aerosol mass concentration was also continuously monitored in each chamber with the in situ design backscattering photosensors. For reasons described in Section 4.1, the aerosol mass concentrations at the outlet of both the solid and liquid aerosol generators were also monitored. An in situ design backscattering sensor was employed at the solid generator outlet. The liquid generator output was monitored with the backscattering sensor without any modifications (see Figure 3-4 for monitoring locations). The sensors

TABLE 4-1. SUMMARY OF SAMPLING METHODS AND FREQUENCIES

System Sampled	Monitoring Method	Frequency
Solid Particulate Aerosol Study		
Chamber aerosol mass concentration	Primary: Gravimetric On-line guide: PCAM sensors	Once per hour of exposure Continuous
Aerosol particle size	QCM cascade impactors[a]	Once per day
Temperature and humidity	Thermo-Hygrometer	Three times per day
Chamber air flow rate	Orifice meter	Continuous
Oxygen	Solid state oxygen detector	Once per day
Solid-liquid Aerosol Mixture Study		
Chamber aerosol mass concentration	Primary: Gravimetric On-line guide: Backscattering photosensor *in situ* design	Once per hour of exposure Continuous
Chamber aerosol particle size	QCM cascade impactors	Once per day
Aerosol mass concentration at generator outlet		
• Solid	Backscattering photosensor *in situ* design	Continuous
• Liquid	Backscattering photosensor regular design	Continuous
Chemical analysis of liquid component of mixture aerosols	HPLC of filter samples	Once per day
Temperature and humidity	Thermo-Hygrometer	Three times per day
Chamber air flow rate	Orifice meter	Continuous
Oxygen	Solid state oxygen detector	Once per day

[a] Particle size distribution of the positive control test aerosol (Cristoballite) was measured with a Mercer Cascade Impactor.

responses were good indicators of short term changes in aerosol concentration and guided the laboratory personnel to make the necessary adjustments in generator settings when concentration excursions were encountered. Records of the sensor outputs were maintained using strip chart recorders.

4.3 AEROSOL PARTICLE SIZE

The aerosol particle size distribution was monitored by a piezoelectric microbalance-based 10-stage cascade impactor. The Quartz Crystal Microbalance (QCM) is a cascade of aerodynamic-intertial impactors (California Measurements, Sierra Madre, CA), in which the suspended particles are classified according to their effective aerodynamic sizes and weighed in situ and in real time on the impaction surface. This is accomplished by using high-frequency, resonating piezoelectric crystals as the impactor plates. A built-in pump samples an aerosol stream at a rate of 0.24 liters/min, separating the aerosol particles into 10 sequential size ranges from 0.05 to 25 μm. Ten audio frequencies, which are proportional to the accumulated mass on the stages, are displayed and printed directly from the instrument. A built-in computer converts the data to the actual mass and size readings. The Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were calculated for each sampling point from the corresponding mass fraction of particles accumulated on each stage of the QCM using a programmable calculator.

Particle size distribution measurements of the highly concentrated aerosols were accomplished with use of a sliding valve as shown in Figure 4-4. The sample is continuously drawn from the chamber. When particle size is to be measured, the slide is pulled out and a slug of the sample is drawn through the QCM. For homogeneity studies of aerosol particle size, samples were collected from the chambers sequentially from the same sampling ports provided for the gravimetric filter sample collection.

For the positive control aerosol, we were not able to measure the particle size with the QCM because the particles did not adhere well to the sensing crystal. Hence, another cascade impactor (Mercer Cascade Impactor, In-Tox Products, Albuquerque, NM) was used to measure the size. The Mercer impactor has six stages and operates at a flow rate of 2 l/min and can measure particles in the range of 0.5 to 12.8 μm diameter. The material collected in each stage was determined gravimetrically.

4.4 CHEMICAL ANALYSIS OF MIXED AEROSOLS FROM FILTER COLLECTED SAMPLES

A high performance liquid chromatography (HPLC) procedure was developed for the chemical analysis of the petroleum-based liquid (PBL) on collected aerosol filter samples with the object of providing the following:

- A simple quantitative analysis of the total amount of PBL aerosol collected on filters
- Qualitative information that the oil fog generation method was not significantly degrading the oil in the resultant aerosol

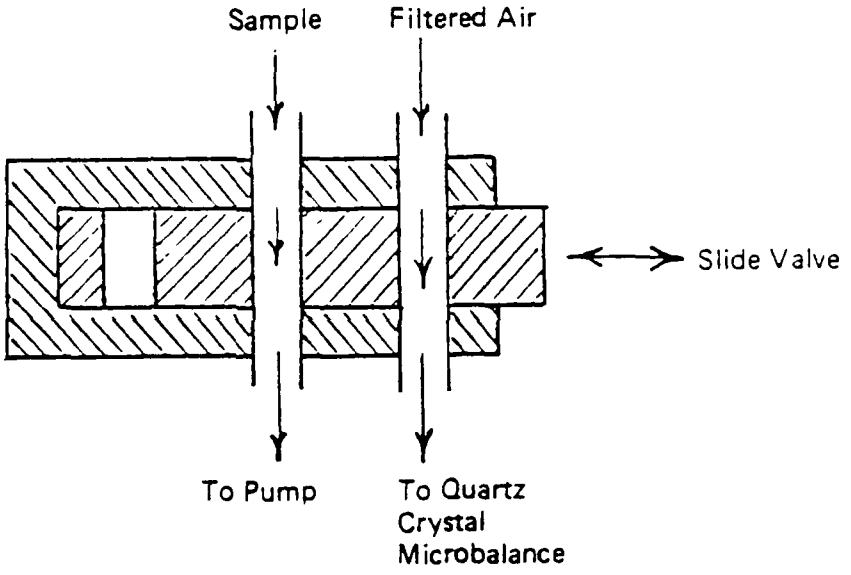


Figure 4-4. High concentration sampling valve for quartz crystal microbalance.

- Qualitative information that the solid component of the aerosol was not significantly degrading the oil in the test atmosphere

4.4.1 Analytical Methods

Samples were analyzed by HPLC, using a Waters system consisting of two Model 6000 A pumps, a Model 740 system controller, a Model UK6 manual injector, and a Model 440 absorbance detector. For quantitative analyses, the oil extracts were eluted on a Sperisorb NH_2 column 4.6 mm ID x 25 cm, using a 98:2 hexane: methylene chloride mixture for five minutes and then programming to 50% methylene chloride in five minutes, holding for five minutes, and continuing to 100% methylene chloride in five minutes. For qualitative analyses, the same solvent systems were used, with the individual programming steps extended to give a total run time of 40 minutes. The solvent flow rate was 2 ml/min. A 50 µl injection of all samples and standards was made, and the constituents of the oil were detected at 254 nm and recorded. The peaks were integrated using a Hewlett Packard Model 3390A recording integrator. The bulk of the oil (95+%) was found to elute in the first five minutes after injection. Additional peaks at later elution times were relatively broad and difficult to integrate; hence for quantitative analyses only those peaks eluting between 0-5 minutes were summed and used for quantitation (concurrent qualitative analyses of filter samples containing PBL aerosol indicated negligible degradation of the contained oil and that quantitative analyses of the samples based on the 0-5 minutes elutions would be valid).

For quantitation of oil samples, a standard curve was generated, based on triplicate analyses of four oil standards in hexane (UV High Purity) and a blank. A linear regression of the resultant average summed peak area vs concentration (mg/ml) was performed. The HPLC analytical method was found to be linear over the concentration range of 0.75 to 9.24 mg/ml oil, with a correlation coefficient of R = 0.9999. Eight replicate injections of a 5.02 mg/ml oil standard gave a precision of 4.84% RSD.

4.4.2 Sample Preparation Procedures

Filter samples containing either oil or oil and particulate are transferred to a 40 ml vial and 25 ml of hexane (UV High Purity) is added. The vials are capped and sonicated in a Bronson water bath sonicator for 20 minutes. The filter is removed with tweezers, rinsed with hexane into the vial and the extract concentrated to approximately 5 ml under a gentle stream of nitrogen. Samples containing particulate and oil are filtered through a 25 mm diameter nylon filter (0.45 µm) with washings and reconcentrated to approximately 5 ml. The samples are quantitatively transferred to either 10, 25, or 50 ml volumetrics, depending on the anticipated concentration of the oil, and diluted to volume with hexane.

4.4.3 Results of Chemical Analysis of PBL Aerosol Collected on Filters

Three filter samples were collected from a chamber test atmosphere containing aerosol generated from the PBL liquid only. These filter samples were analyzed, within 24 hrs of collection, by HPLC to determine their PBL content for comparison against the gravimetrically determined values of PBL. Data from these analyses are presented in Table 4-2. Prior to the HPLC

analyses a recovery factor for the PBL from spiked filters (pure PBL) was determined at 88.0±3.6%. Application of this recovery factor to the HPLC analyses resulted in an overestimation of the mass of PBL on the filters (results shown in column HPLC[c] of Table 4-2) compared to the gravimetrically determined values, suggesting that possibly the filter PBL recovery factor was in error. A second determination of the recovery factor was made using a slightly modified experimental approach to spiking the filters and a value of 94.3±5.94% was obtained. Application of this recovery factor to the HPLC analyses slightly improved the agreement between the two measurements, but still resulted in an overestimation of the mass of PBL on filters (results shown in column HPLC[d] of Table 4-2).

TABLE 4-2 HPLC AND GRAVIMETRIC ANALYSES OF PBL AEROSOL ON FILTER COLLECTED SAMPLES

Sample No.	Mass of Aerosol on Filter[a] (mg) Gravimetric	HPLC[b]	HPLC[c]	HPLC[d]
1	185.2	181	206	192
2	169.1	166	189	176
3	176.3	167	190	177

[a] Glass fiber filter
[b] Mass of PBL aerosol on filter analyzed by HPLC
[c] Mass of PBL aerosol on filter analyzed by HPLC and corrected for recovery factor of 88.0%
[d] Mass of PBL aerosol on filter analyzed by HPLC and corrected for recovery factor of 94.3%

It can be seen from these data that if these recovery factors are used, the PBL aerosol contents of the filters determined by the HPLC analyses are consistently higher than those determined gravimetrically. If the true HPLC recovery factor were ≈100%, instead of the lower values shown above, a better agreement between the two measurements would result (see column HPLC[b] of Table 4-2). This and the variation in the recovery factors obtained suggest that this facet of the analysis may be in error and therefore, will be repeated prior to the exposure studies with aerosol mixtures.

4.4.4 Qualitative Analyses of Filter Collected PBL Aerosol Samples

Qualitative analyses were made by HPLC on filter-collected aerosol samples obtained during generation of chamber test atmospheres containing pure PBL overall and a mixture of PBL and solid particulate aerosol (Ratio PBL aerosol: solid particulate aerosol ≈5) with the object of determining if degradation of the PBL occurred due to the generation process or to contact of the PBL with the solid particulate aerosol. Chromatograms of the pure PBL, the PBL aerosol extracted from filter-collected samples, and PBL extracted from a filter containing both PBL and solid particulate aerosol generated simultaneously are presented in Figures 4-5 A, B, and C. Qualitatively these chromatograms are not significantly different; we concluded that degradation of the PBL during generation of the aerosol or by its subsequent contact with the solid particulate aerosol is negligible. This finding indicates that HPLC

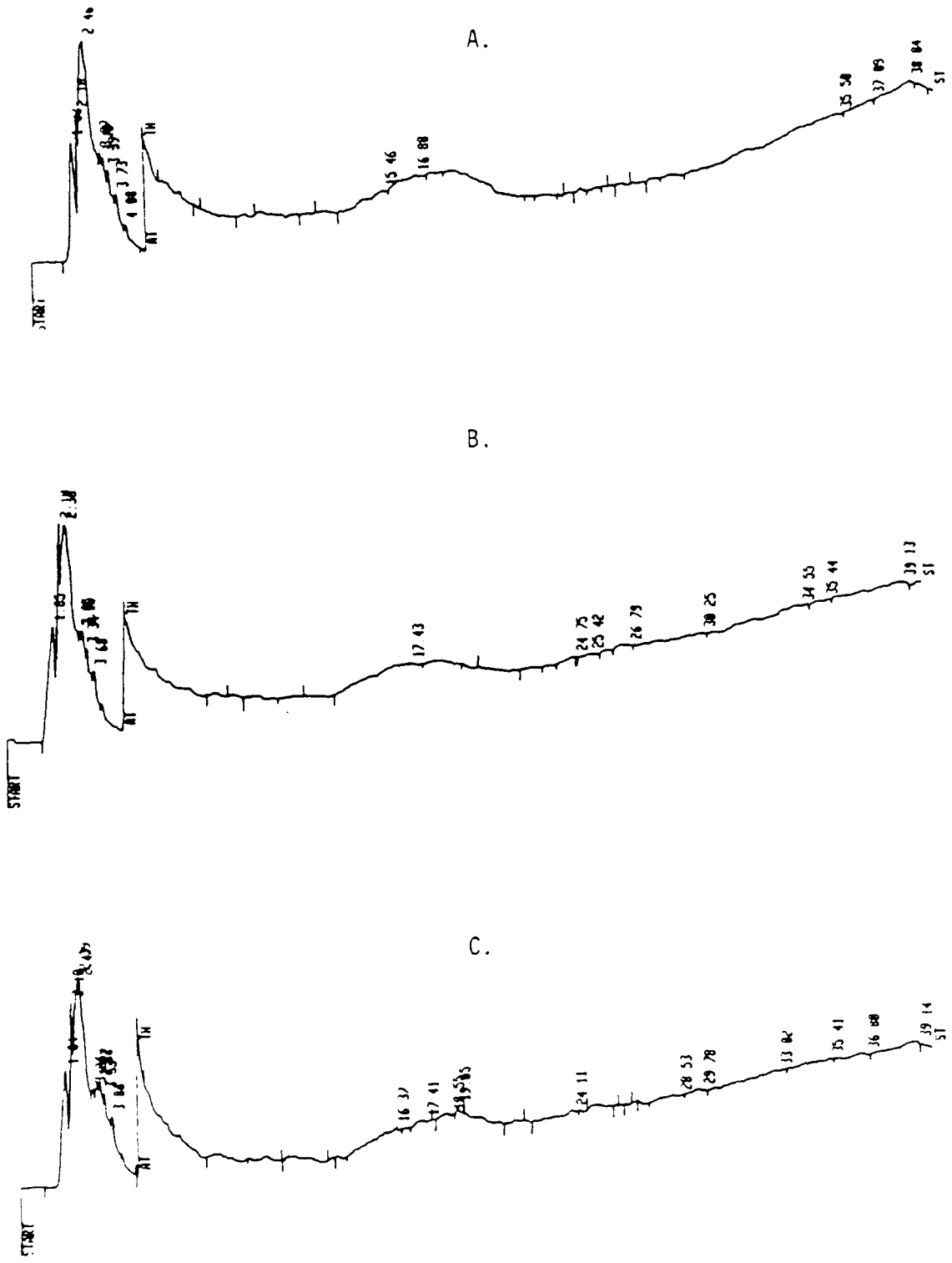


Figure 4-5. Chromatograms of PBL aerosol samples: (A) pure PBL, (B) PBL aerosol extracted from filter, and (C) PBL extracted from filter containing both PBL and solid particulate aerosol generated simultaneously.

analyses of filter samples containing both PBL and solid particulate aerosol are a viable way for determining the PBL content of the aerosol mixtures.

4.5 OXYGEN MONITORING

A commercial oxygen analyzer monitors the oxygen concentration in the chamber atmosphere. An integral pump draws gas through the instrument at a predetermined rate and a solid state oxygen detector senses the oxygen in the gas stream. The instrument readout is presented as percentage of oxygen in the sample gas stream with the range spanning 0 to 25 percent. Prior to use, the analyzer is allowed to "warm up" for 30 minutes to stabilize. The oxygen concentration in the chamber was always above the required lower limit of 19%.

4.6 TEMPERATURE AND HUMIDITY

Temperature and humidity in the chamber and in the laboratories are measured by a thermohygrometer (Cole Parmer, Model 3309-60). The hygrometer operates on the principle of thin-film capacitance. The hygrometer's probe has a perforated plastic cap to protect the humidity sensor from dusty environments while allowing good air re-cirulation. The temperature and humidity in the inhalation laboratories and the chamber were within the required limits of 24-27°C and 40 to 60% RH respectively.

5. AEROSOL HOMOGENEITY STUDIES

5.1 APPROACH

The objective of these studies was to evaluate the spatial and temporal homogeneity of the chamber atmosphere through a procedure of simultaneous sampling. Two separate homogeneity studies were conducted: the first for the solid particulate aerosols and the second for the solid-liquid aerosol mixtures. A total of five exposure chambers were used for each of the homogeneity studies. One of the chambers, Chamber #3, was randomly selected as the Pilot Chamber. Chamber #4 was assigned exclusively for the positive control material. For the Pilot Chamber, sufficient numbers of sampling points were selected to allow for characterization of the spatial aerosol homogeneity within the chamber along with a series of sequential samples that were taken from multiple randomly-selected fixed points to define temporal homogeneity for a period corresponding to the duration of the longest exposure (planned to be ≤4 hours). The aerosols were monitored for mass concentration and particle size at three or four generator settings (aerosol concentrations), replicating all tests at one generator setting three times.

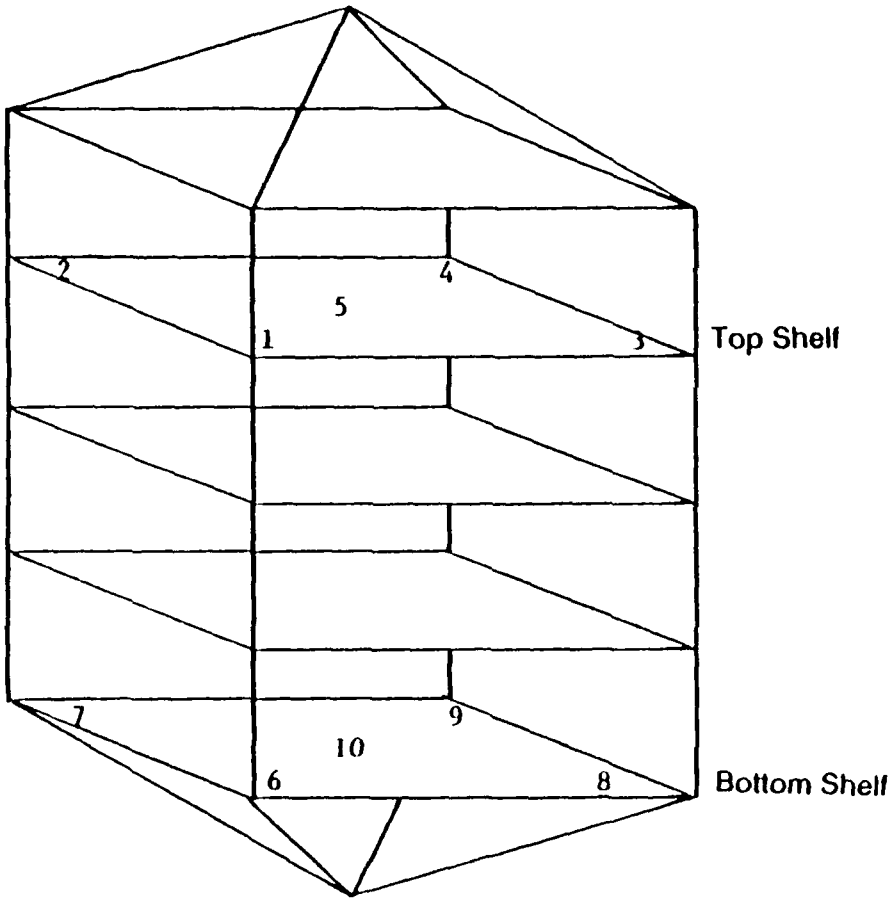
After standardization of the Pilot Chamber was completed, a single generator setting was randomly selected for three of the four remaining chambers. The fourth chamber, as stated earlier, was tested with positive control material. Spatial and temporal homogeneity tests for the above mentioned aerosol parameters were conducted.

The ultimate objective was to reduce the variability of spatial and temporal homogeneity, with appropriate chamber modifications if necessary, to ± 20 percent of the mean of each parameter throughout the chambers and range of concentrations tested. All the aerosol homogeneity studies were conducted with animal cages in place.

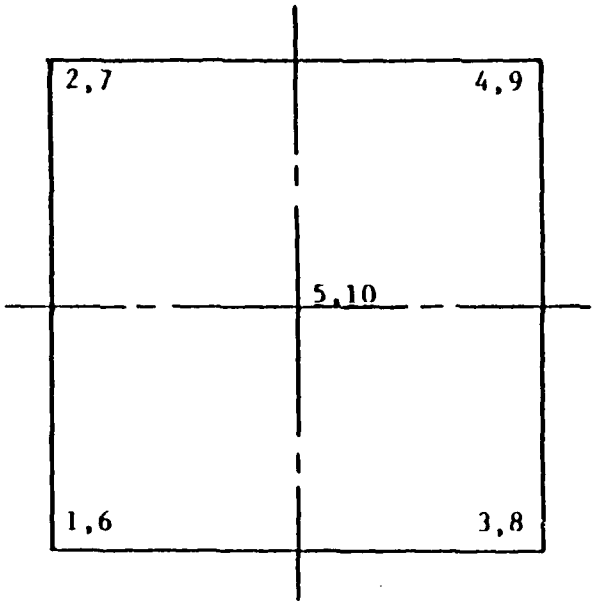
5.2 PILOT CHAMBER (CHAMBER #3)

For the Pilot Chamber homogeneity studies, a sampling schedule for ten (10) locations in a three-dimensional array of points (Figure 5-1) was designed on aerosol physical considerations.

To facilitate uniform access of the sampling probes for homogeneity testing into the chambers, an acrylic plastic panel was fitted to replace the front door for the duration of the homogeneity studies. Ten 98 cm long stainless steel tubes were positioned so that both the top and bottom shelves had two tubes set at each side, plus one at the center, for a total of five tubes per shelf (Figure 5-2). The tubes at each shelf level entered at approximately the middle of the cage height (animal breathing zone) and protruded into the chamber for a distance of 7.5 cm and 82.5 cm respectively. This design assured that the aerosol samples always traveled the same distance from the sampling point to the collecting filters which were attached to the outside end of each tube. Aerosol samples for measurement of particle size also were taken from these locations.



A. Isometric diagram of chamber.



B. Overhead view of shelf.

Figure 5-1. Sampling point locations for Aerosol Homogeneity Tests.

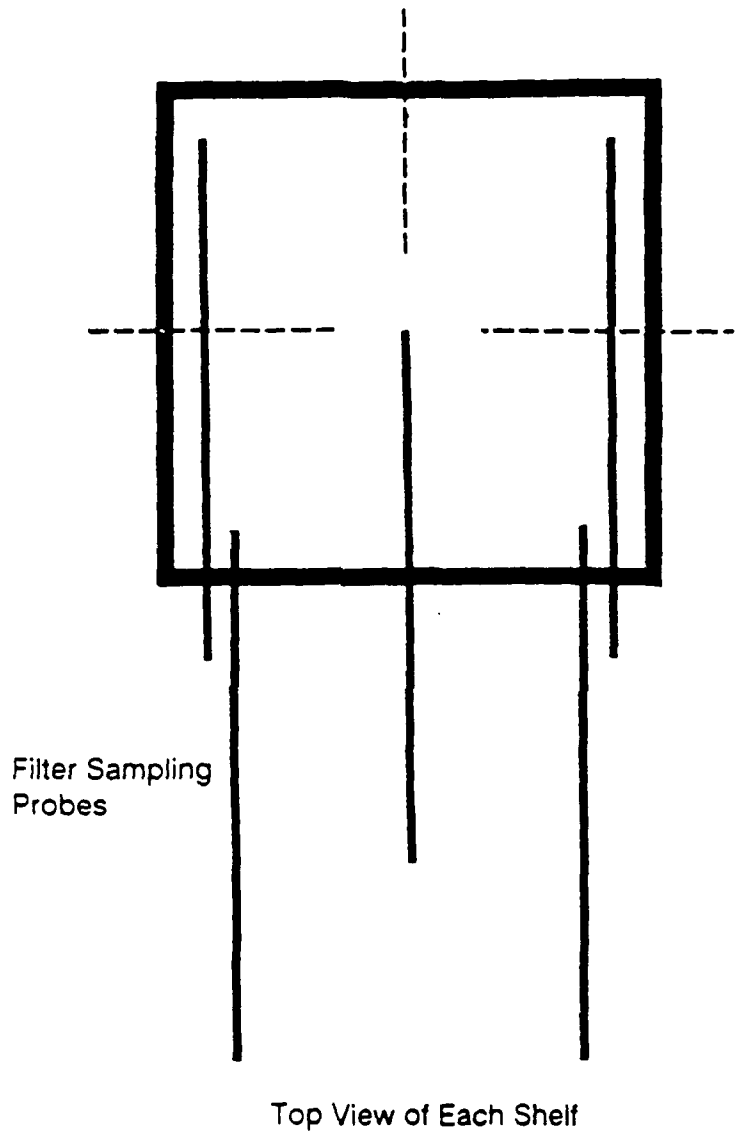


Figure 5-2. Sampling probe locations for Aerosol Homogeneity Studies.

The sampling methods used to determine the aerosol mass concentration, and aerosol particle size were previously described (the mass median aerodynamic diameter MMAD, was used for testing spatial and temporal homogeneity of particle size). Similarly, measurements of temperature and relative humidity of the conditioned air and the oxygen levels in the exposure chambers have been also discussed (Section 4 Test Atmosphere Monitoring).

Aerosol homogeneity tests were conducted in the Pilot Chamber at 10 locations per concentration, at four concentrations for solid aerosols, and at three concentration combinations for mixed aerosols with three replicate experiments at one of these concentrations for each aerosol type. One of the three combinations for the mixed aerosol study required just the PBL aerosol by itself without the solid particulate aerosol.

The test concentrations selected by the government were:

- Solid Particulate Aerosols : 10, 60, 100, and 200 mg/m^3 (one replicate at 10, 60, and 200, three replicates at 100 mg/m^3)
- Solid/Liquid Aerosol Mixture : 0/1000, 200/1000, 200/500 mg/m^3 (one replicate at 0/1000 and 200/1000, three replicates at 200/500 mg/m^3)

In addition, the homogeneity of the cristobalite particulate aerosol (positive control) was tested at the 200 mg/m^3 level.

The concentrations were adjusted to these specified levels by using generator settings established in previous exploratory experiments. The settings were maintained at a constant level for each of the replicate tests, at a given concentration. Temperature and relative humidity were monitored routinely and maintained in the specified ranges of 24 to 27°C and 40 to 60% RH.

The spatial and temporal homogeneity of the aerosols in the chamber were determined by the following sampling procedure: Data were collected in two sets. The first set contained six randomly-selected sampling locations. The second set contained the remaining four locations plus one location from the first set. Filter and particle size samples were collected once per hour for four hours for all the sampling locations within the set. At the end of four hours, the second set of sampling locations was monitored (this represents one replicate experiment).

The randomization pattern was maintained at a constant level for all three concentrations. Thus, the Pilot Chamber study produced 44 (11 samples/hour x 4 hours) filter collected samples and particle size data per replicate experiment for the evaluation of spatial and temporal homogeneity at each concentration level.

5.3 REMAINING CHAMBERS

Each of the remaining three chambers was assigned an aerosol concentration level (for both the solid and the solid-liquid aerosol mixture) randomly selected from those used in the Pilot Chamber studies (because only two concentration combinations were used for the solid-liquid mixture , one of

these was repeated in two chambers). The same procedure used for Pilot Chamber studies was used to establish the homogeneity in the remaining chambers. In addition, inter-chamber comparisons were made. The overall mean for each parameter for each chamber was compared to the overall mean of the Pilot Chamber for that respective concentration level. Table 5-1 presents the concentration assignments for all the chambers for both the solid and the solid-liquid aerosol mixture studies.

5.4 STATISTICAL EVALUATION OF AEROSOL HOMOGENEITY MEASUREMENTS

The aerosol concentration and MMAD calculated from the raw data were entered onto floppy disks for transmittal to R. Gibbons, Statistical consultant, for analysis. Prior to transmittal, these data were subjected to 100% internal quality control. A complete listing of these data is also attached in Appendix B. All raw data recorded during the course of the Phase I studies will be retained in the IITRI Life Science's archives and will be available for inspection.

The aerosol concentration and MMAD were analyzed using an analysis of variance model and estimates of various components of variance were obtained. A statistical analysis report detailing the methodology and results is attached in toto in Appendix C. Only a summary of the findings is presented in this section. The primary purpose of these analyses was to estimate the amount of spatial and temporal variability within the chambers and to determine if these estimated variances represented significant variation about the estimated mean value of the chamber. For this determination, a criterion of 20% of the estimated mean value was used; if the variance estimate accounted for 20% or more of the variation about the estimated mean, it was deemed to be significant. Finally, inter-chamber analyses were performed to determine if significant variation was attributable to the design and operation of the chamber and to examine the estimated spatial and temporal variation across chambers.

5.4.1 Homogeneity Study of the Solid Particulate Aerosol

Within Chamber Analysis: The results from the variance component analyses are given in Tables 5-2 (for aerosol concentration) and 5-3 (for particle size given as MMAD) of the aerosol size distribution. In all cases the shelf x time interaction was non-significant and was not included in the final tables. As can be seen from Table 5-2, the coefficient of variation for the aerosol concentration never exceeds 20% in any of the chambers at any target concentration. Additionally, the means for the aerosol concentrations are usually very close to the target concentrations and always within one standard deviation of the target value.

For particle size (MMAD), given in Table 5-3, all of the coefficients of variation exceed 20%. However, the percentage of variation due to the shelf or time effects never exceeds 20%, and the bulk of the variance is attributable to the residual component, which combines all other effects, such as inhomogeneity in the bulk test material and monitoring instrument response factor variation. The variations observed in particle size, even though statistically significant, are not expected to be biologically meaningful in

TABLE 5-1 CHAMBER NUMBER VS TEST CONCENTRATION

Chamber Number	Solids Test Concentration (mg/m^3)	Solid/Liquid Mixture Test Concentration (mg/m^3)
1	60	200/500[a]
2	100	200/500[a]
3 (Pilot)	10, 60, 100[b], 200	200/500[b], 0/1000, 200/1000
4 (Positive Control)	200	ND[c]
5	10	200/1000

a There were only two selected concentrations for the solid-liquid aerosol mixtures, therefore, two chambers were tested at one concentration level.
b Replicated three times at one generator setting.
c Chamber #4 was set up for the positive control particulate aerosol.

TABLE 5-2

Components of Variance for the Solid Particulate Aerosol Concentrations (mg/m^3) in the Exposure Chambers (Within Chamber Analysis)

n	mean	standard deviation	coefficient of variation	Variance Components rep	set	shelf	time	residual
pilot chamber - target concentration = 10 mg/m^3								
44	10.60	1.83	17.3%		0.8%	0.2%	13.1%	3.3%
pilot chamber - target concentration = 60 mg/m^3								
44	58.70	7.12	12.1%		0.6%	3.2%	3.2%	5.1%
pilot chamber - target concentration = 100 mg/m^3								
131[a]	103.01	15.85	15.4%	0.9%	5.5%	4.9%	0.2%	3.9%
pilot chamber - target concentration = 200 mg/m^3								
44	197.25	17.95	9.1%		2.5%	3.9%	0.2%	2.5%
chamber 5 - target concentration = 10 mg/m^3								
44	9.47	1.87	19.8%		0.3%	8.1%	2.3%	9.1%
chamber 1 - target concentration = 60 mg/m^3								
43	58.86	7.38	12.5%		2.3%	5.2%	2.4%	2.6%
chamber 2 - target concentration = 100 mg/m^3								
44	92.65	9.45	10.2%		1.6%	3.8%	1.5%	3.4%

[a] The Pilot Chamber study at this aerosol mass concentration was replicated three times

TABLE 5-3

Components of Variance for the Solid Aerosol Particle Size (MMAD in μm) in the Exposure Chambers (Within Chamber Analysis)

		standard	coefficient	*Variance Components*				
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 10 mg/m³								
44	1.13	0.48	42.5%		0.0%	12.5%	15.1%	14.9%
pilot chamber - target concentration = 60 mg/m³								
20[a]	1.59	0.75	47.2%			0.0%	2.1%	45.1%
pilot chamber - target concentration = 100 mg/m³								
85[b]	1.55	0.65	41.8%	0.0%	1.1%	2.8%	14.7%	23.2%
pilot chamber - target concentration = 200 mg/m³								
44	1.96	0.51	26.0%		1.6%	0.3%	12.5%	11.6%
chamber 5 - target concentration = 10 mg/m³								
43	1.45	0.34	23.4%		0.8%	5.4%	0.3%	16.9%
chamber 1 - target concentration = 60 mg/m³								
43	1.86	0.55	29.7%		10.4%	0.0%	8.4%	10.9%
chamber 2 - target concentration = 100 mg/m³								
44	1.50	0.43	28.7%		0.0%	1.1%	6.5%	21.1%

[a] Reduced number of data due to QCM malfunction.
[b] The Pilot Chamber study for particle size was replicated two times.

terms of inhalation and deposition of particles because the actual values for the MMAD were well within the respirable range (Dennis, W. L. "Inhaled Particles and Vapours", Oxford: Pergamon Press, 1961). Due to a malfunction in the QCM, no particle size data were collected during the third replication of 100 mg/m^3 target level and the number of observations was reduced to 20 at the 60 mg/m^3 target level both in the Pilot Chamber.

Inter-Chamber Comparison: For each test concentration, data from the Pilot Chamber were compared with the data from one of the remaining chambers with the same test concentration. In general, a total of 88 observations will be available (44 each for the Pilot and for the chamber in question) to assess the chamber variability and the homogeneity of the time and shelf effects across chambers. For concentration levels where Pilot Chamber data from more than one replication were available, a single replication was selected at random for comparison. The results from these variance component analyses are given in Tables 5-4 (for aerosol concentration) and Table 5-5 (for particle size). The coefficient of variation for aerosol concentration, listed in Table 5-4 does not exceed 20% for any of the three target concentrations. Furthermore, the variability due to the interactions of chamber x time and chamber x shelf is small, indicating that the low degree of spatial and temporal variability is consistent across chambers. The overall means for the actual concentrations are very close to the target concentrations and always within one standard deviation of the target value.

For particle size, given in Table 5-5, all of the coefficients of variation exceed 20%; however, the percentage of variation due to any of the model effects never exceeds 20%. Again, the actual values for MMAD in these chambers were well within the respirable range.

Positive Control Particulate Aerosol: Homogeneity of concentration for the positive control aerosol (silica in the form of Cristobalite) chamber (chamber #4), shown in Table 5-6, was obtained at the test concentration of 200 mg/m^3. The concentration data has a mean of 179.55 with a standard deviation of 32.45 and a coefficient of variation of 18.07%. From the components of variance analysis, the computed percentage of variation for the set, shelf, and time effects are 0.6%, 13.2%, and 2.6%, respectively. The residual variability accounted for 1.7% of the overall variation. Because the shelf x time interaction was non-significant, it was not included in the final model. Thus, the coefficient of variation for aerosol concentration does not exceed 20% and the mean for the actual concentrations is easily within one standard deviation of the target value.

Due to the technical difficulties (described in Section 4) encountered in measuring the particle size of the positive control aerosol with the QCM, we used a Mercer Cascade Impactor to measure the particle size distribution of the positive control aerosol. Typically, the sampling times required with the Mercer Cascade Impactor are in the order of 40 minutes as opposed to less than a minute with the QCM.

In view of these long sampling times for particle size, it was not possible to obtain sufficient data to perform the components of variance model. Instead, to estimate a coefficient of variation for the temporal effect, one sample per hour for four hours was obtained from a reference

TABLE 5-4

Components of Variance for the Solid Particulate Aerosol Concentrations (mg/m^3) Between Various Exposure Chambers (Inter-Chamber Comparison)

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 10 mg/m^3										
88	10.03	1.93	19.2%	0.0%	1.5%	1.8%	0.2%	5.6%	3.4%	6.7%
target concentration = 60 mg/m^3										
87	58.78	7.21	12.3%	0.0%	4.8%	1.0%	0.0%	2.1%	0.0%	4.3%
target concentration = 100 mg/m^3										
87[a]	95.71	13.18	13.8%	0.0%	5.8%	0.8%	0.9%	0.4%	0.7%	5.2%

[a] Comparison performed with the data from the first replication within the Pilot Chamber.

TABLE 5-5

Components of Variance for the Solid Aerosol
Particle Size (MMAD in μm) Between Various Exposure Chambers
(Inter-Chamber Comparison)

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 10 mg/m³										
87	1.29	0.45	34.5%	0.0%	6.6%	0.0%	5.9%	9.2%	0.9%	11.9%
target concentration = 60 mg/m³										
40[a]	1.58	0.59	37.3%		0.0%	9.7%	0.0%	0.0%	0.0%	27.6%
target concentration = 100 mg/m³										
85[b]	1.56	0.69	44.2%	0.0%	1.1%	13.0%	0.0%	0.4%	0.0%	29.6%

a Due to a malfunction of the QCM only 20 samples were collected and compared with the corresponding 20 samples of the Pilot Chamber.

b Comparison performed with data from the second replication within the Pilot Chamber.

TABLE 5-6

Components of Variance for the Aerosol Concentrations (mg/m^3) of Positive Control Material in the Exposure Chambers (Within Chamber Analysis)

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
chamber 4 - target concentration = 200 mg/m³								
44	179.55	32.45	18.1%		0.6%	13.2%	2.6%	1.7%

port. For these data, the mean of 3.13 with a standard deviation of 0.19 yields a coefficient of variation of roughly 6%. Similarly, to estimate a coefficient of variation for the spatial effect, one sample per port was collected. The data have a mean of 3.00 with a standard deviation of 0.21 which corresponds to a coefficient of variation of approximately 7%. Thus, from these data, we can conclude that no appreciable effects due to shelf or time were observed for particle size.

5.4.2 Homogeneity Study of the Solid-Liquid Aerosol Mixture

Data from the test concentrations of 700 (200 solid/500 liquid), 1000 (no solid/1000 liquid), and 1200 (200 solid/1000 liquid) mg/m^3 were obtained within the Pilot Chamber (Chamber #3), while data from Chambers 1 and 2 corresponded to the target concentration of 700, and data from Chamber 5 corresponded to the target concentrations of 1200. No data were collected from Chamber #4, using mixed aerosols, because it was dedicated to positive control material. For the target concentration of 700 in the Pilot Chamber, data were collected at three replications.

Within Chamber Analysis: The results from the variance component analyses are given in Tables 5-7 (for aerosol concentration) and 5-8 (for particle size). In all cases, the shelf x time interaction was non-significant, and so was not included in the final tables. As can be seen from Table 5-7, the coefficient of variation for aerosol concentration is typically small and never exceeds 20% in any of the chambers at any target concentration. The means for the actual concentrations are usually very close to the target concentrations and always within one standard deviation of the target value.

The particle size, given in Table 5-8, show that all of the coefficients of variation exceed 20% as was the case with solid particle. However, the percentage of variation due to the time effect never exceeds 5%, while the percentage of variation attributable to the shelf effect exceeds the 20% cutpoint only in Chamber #1. Furthermore, the actual values for the MMAD in these chambers are well within the respirable range and these variations are expected to be of no significance from a biological point of view.

Inter-Chamber Comparison: Inter-chamber analysis was performed to assess the homogeneity of the time and shelf effects across the chambers.

The results from these variance component analyses are given in Tables 5-9 (for aerosol concentration) and 5-10 (for particle size). The coefficient of variation for actual concentration, listed in Table 5-9, does not exceed 20% for either of the two target concentrations. Furthermore, the variability due to the interactions of chamber x time and chamber x shelf is very small, indicating that the low degree of spatial and temporal variability is consistent across chambers. The overall means for the actual concentrations are very close to the target concentrations and always within one standard deviation of the target value.

For particle size, given in Table 5-10 both of the coefficients of variation exceed 20%; however, the percentage of variation due to any of the

TABLE 5-7

Components of Variance for the Solid-Liquid Mixture Aerosol Concentrations (mg/m^3) in the Exposure Chambers (Within Chamber Analysis)

n	mean	standard deviation	coefficient of variation	Variance Components: rep	set	shelf	time	residual
pilot chamber - target concentration = 700								
121	706.51	54.46	7.7%	1.7%	0.0%	2.0%	0.0%	4.0%
pilot chamber - target concentration = 1000								
44	1025.1	36.95	3.6%		0.3%	1.6%	0.0%	1.7%
pilot chamber - target concentration = 1200								
44	1181.0	122.6	10.4%		2.2%	0.2%	2.7%	5.4%
chamber 1 - target concentration = 700								
44	684.82	34.17	5.0%		0.1%	2.1%	0.4%	2.4%
chamber 2 - target concentration = 700								
44	734.82	52.35	7.1%		0 2%	3.1%	0.6%	3.2%
chamber 5 - target concentration = 1200								
44	1166.4	65.51	5.6%		0.2%	1.2%	2.2%	2.0%

Target Concentrations:
700 mg/m^3 (200 solid/500 liquid)
1000 mg/m^3 (no solid/1000 liquid)
1200 mg/m^3 (200 solid/1000 liquid)

TABLE 5-8

Components of Variance for the Solid-Liquid Mixture Aerosol Particle Size (MMAD in μm) in the Exposure Chambers (Within Chamber Analysis)

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 700								
110	0.37	0.14	37.8%	2.3%	0.0%	12.2%	0.0%	23.2%
pilot chamber - target concentration = 1000								
40	0.35	0.09	25.7%		0.0%	13.9%	1.0%	10.8%
pilot chamber - target concentration = 1200								
40	0.40	0.11	27.5%		0.0%	5.6%	3.8%	18.2%
chamber 1 - target concentration = 700								
40	0.29	0.10	34.5%		0.0%	23.8%	1.1%	9.6%
chamber 2 - target concentration = 700								
40	0.34	0.11	32.4%		0.0%	12.1%	0.0%	20.3%
chamber 5 - target concentration = 1200								
40	0.39	0.08	20.5%		0.0%	8.0%	1.1%	11.4%

TABLE 5-9

Components of Variance for the Solid-Liquid Mixture Aerosol Concentrations (mg/m^3) Between Various Exposure Chambers (Inter-Chamber Comparison)

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 700										
132[a]	705.39	49.13	7.0%	0.0%	2.4%	0.0%	1.5%	0.3%	0.0%	2.8%
target concentration = 1200										
88	1173.7	98.00	8.3%	1.1%	0.6%	2.2%	0.0%	0.2%	0.0%	4.2%

[a] Comparison performed with data from the second replication within the Pilot Chamber.

TABLE 5-10

Components of Variance for the Solid-Liquid Mixture Aerosol Particle Size (MMAD in μm) Between Various Exposure Chambers (Inter-Chamber Comparison)

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 700										
120[a]	0.35	0.12	34.3%	0.2%	14.5%	0.0%	6.8%	0.7%	0.0%	12.1%
target concentration = 1200										
80	0.39	0.10	25.6%	0.0%	6.9%	2.0%	0.0%	0.8%	0.0%	15.8%

[a] Comparison performed with data from the second replication within the Pilot Chamber.

model effects never exceeds 20%. In light of the finding that the shelf effect of Chamber #1 exceeded 20%, it is important to note that the overall variability due to the shelf effect at the concentration of 700 mg/m^3 is below 20%.

5.4.3 Summary Findings

The aerosol concentration variations within the Pilot Chamber and between chambers never exceeded 20% for both the solid, as well as the solid-liquid aerosol mixture. The estimated mean concentrations were always within a standard deviation of the target value. For particle size, the estimated coefficient of total variations exceeded 20% on several occasions but the variability due to time or shelf was always below 20%, indicating that the size distribution was homogeneous with respect to time and space. Moreover, absolute changes in particle sizes are quite small and will have no effect on the deposition locations of these particles in the lung.

6. CONCLUSIONS

This Phase I report summarizes test atmosphere development and aerosol homogeneity studies for aerosols of a solid particulate test material and mixtures of this solid particulate aerosol with a petroleum-based liquid aerosol.

Two aerosol generators (provided by the Government through ORNL) were used to generate the solid particulate aerosols and the liquid (PBL) aerosols respectively. Aerosols of the solid test material are generated by pneumatic re-dispersion of the bulk solid using a jet mill, a screw feeder, and compressed air. The particles produced enter a size classifier and, if small enough, exit the system as aerosol. Large particles stay in the mill until their size is reduced enough to pass through the classifier. For concentrations below 100 mg/m^3, a slip stream dilution system was employed. Aerosols of the PBL are generated by injecting it onto a temperature-controlled Vycor glass heating element in nitrogen atmosphere. The PBL flash evaporates and condenses to form aerosol when mixed with dilution air. For generating the solid-liquid aerosol mixtures, the solid aerosol and the PBL aerosol are dynamically mixed before entering the chambers. HPLC analyses of the PBL aerosol collected on the filters revealed that the PBL did not degrade either during the generation process or when mixed with solid particulate aerosol.

The solids aerosol generator was tested within the concentration range of 10 to 200 mg/m^3 and the PBL aerosol generator in the range of 500 to 1000 mg/m^3 as required. For both generation systems, aerosol outputs remained stable at these concentration levels and we believe that the performance will be similar at any other concentration levels within the tested ranges. Generator settings may need some readjustment for other aerosol concentrations which may be used in the upcoming exposure studies.

Three real time optical aerosol sensors were evaluated for monitoring the solid as well as solid-liquid aerosol mixtures. The extreme stickiness of the solid-liquid aerosol mixtures combined with high electrostatic charge of the solid aerosol produced particle deposition onto the sensor's optics and was a major operational problem. Sensors required cleaning within an hour or two of operation. Hence, gravimetric filter collection was chosen as the primary method for determining the chamber aerosol concentrations. The real time sensors were used as on-line guides for generator adjustment.

For studies with solid particulate aerosol only, a commercially available sensor, the portable continuous aerosol monitor (PCAM) was used. For studies with solid-liquid aerosol mixtures, the concentration was monitored at three locations: at the solid aerosol generator outlet, the liquid (PBL) aerosol generator outlet, and in the chamber itself. We modified the ORNL photosensor, to provide a protective air sheath, and it was used at the solid generator outlet and in the chamber ("in situ sensor"). The output from the liquid aerosol generator was monitored with the original ORNL photosensor.

Particle size distribution of the solid, and solid-liquid aerosol mixtures was determined by a Quartz Crystal Microbalance (QCM)-based cascade impactor. The MMAD ranges for the solid particulate aerosol was 1.5 to 2.0 μm, and for the solid-liquid aerosol mixture 0.3 to 0.4 μm (for ratio of solid to liquid in the aerosol mixtures, see below). The MMAD of the positive control material measured with a Mercer Cascade Impactor, was approximately 3.0 μm.

Spatial and temporal homogeneity of the chamber test atmospheres was established through a procedure of simultaneous sampling from several locations within the chambers. Aerosol mass concentration and particle size were measured at each concentration as a function of location and time. Two homogeneity studies were conducted: the first for the solid particulate aerosols, and the second for the solid-liquid aerosol mixtures. The positive control material was tested as part of the solid particulate homogeneity studies. The test concentrations were:

Solid particulate aerosols (mg/m^3) : 10, 60, 100, 200
Solid/liquid aerosol mixtures (mg/m^3) : 200/500, 200/1000, 0/1000
Positive control aerosol (mg/m^3) : 200

The homogeneity data collected were statistically analyzed to establish the total and individual components of variance, such as effects due to time, shelf, chamber, etc. (A comprehensive statistical analysis report is attached in toto in Appendix C).

Results of the statistical analyses revealed that the total variation of the aerosol concentration was within the required 20% limits for all the concentration levels tested. For spatial homogeneity, the variation attributable to shelf was always less than 10% for both the solid and solid-liquid aerosol mixtures. For temporal homogeneity, the variation was also less than 10% at all test runs except once (at the 10 mg/m^3 level of the solid aerosol only study). For the inter-chamber comparison, the aerosol concentration variations between exposure chambers were below 5% at all concentration levels.

For aerosol particle size, the data showed the total variations to exceed 20% most of the time; however, the mean particle sizes were always within the inhalable range. In addition, variations attributable either to shelf or time were always less than 20%, indicating that these variations are not due to an inhomogeneity with respect to time or location. Moreover, the MMAD values were well within the respirable range of Practical sizes and therefore these variations are expected to have no significance from the biological point of view.

Based on these studies, it is concluded that adequate levels of spatial and temporal homogeneity were attained for the aerosol concentration and particle sizes in all the chambers and at all the target concentration levels tested.

APPENDIX A

SOLIDS FEEDER CALIBRATION

FEED RATE OF ACCURATE (MODEL 100) SCREW FEEDERS
1/4" SCREW AND CENTER ROD
USING SOLID TEST MATERIAL

Feeder No/Chamber	Setting	Revolution Per Minute	Feed Rate (g/min)
1/Chamber #1	1.0		0.03
	1.5	5.2	0.16
	2.0	13.1	0.23
	2.5		0.31
	3.0		0.36
2/Chamber #2	1.0		0.04
	1.5	4.8	0.16
	2.0	10.5	0.22
	2.5		0.30
	3.0		0.34
3/Chamber #3 (Pilot)	1.0		0.07
	1.5	7.2	0.23
	2.0	15.5	0.34
	2.5		0.45
	3.0		0.51
4/Chamber #4 (Positive Control)	1.0	3.5	0.21[a]
	1.5		0.48
	2.0	12.2	0.61
	2.5		0.92
	3.0		1.06
5/Chamber #5	1.0		0.05
	1.5	6.0	0.18
	2.0	12.5	0.25
	2.5		0.36
	3.0		0.41

[a] Feed rate measured with positive control material (Cristobalite)

APPENDIX B

HOMOGENEITY DATA TABLES

TABLE B1: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 200 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	214	1.66
8	2	1	1	182	1.05
3	1	1	1	200	1.82
2	1	1	1	213	1.59
10	2	1	1	182	1.82
6	2	1	1	205	1.49
7	2	1	1	192	1.35
4	1	1	1	192	2.14
11	2	1	1	156	1.05
5	1	1	1	195	1.59
9	2	1	1	175	0.76
1	1	2	1	219	1.78
8	2	2	1	218	1.87
4	1	2	1	199	1.32
2	1	2	1	217	1.88
10	2	2	1	181	2.17
6	2	2	1	188	1.43
7	2	2	1	201	2.51
3	1	2	1	208	1.78
11	2	2	1	178	1.87
5	1	2	1	198	1.77
9	2	2	1	189	0.63
8	2	3	1	190	2.12
1	1	3	1	226	2.18
4	1	3	1	201	2.37
2	1	3	1	219	2.45
10	2	3	1	193	2.18
6	2	3	1	174	2.45
7	2	3	1	207	3.15
3	1	3	1	216	2.07
11	2	3	1	183	2.12
5	1	3	1	212	2.14
9	2	3	1	197	1.86
3	1	4	1	193	2.81
10	2	4	1	170	2.22
1	1	4	1	238	2.25
11	2	4	1	168	2.36
2	1	4	1	231	2.19
5	1	4	1	181	2.16
6	2	4	1	218	2.29
7	2	4	1	184	2.42
4	1	4	1	182	2.11
8	2	4	1	199	2.36
9	2	4	1	195	2.44

ND= NO DATA

TABLE B2: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 100 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
6	2	1	3	118.0	1.09
2	1	1	3	126.0	1.42
8	2	1	3	112.0	1.12
1	1	1	3	126.0	1.71
9	2	1	3	115.0	1.09
8	2	2	3	84.4	1.12
2	1	2	3	96.0	1.50
9	2	2	3	89.2	1.06
6	2	2	3	92.0	1.13
1	1	2	3	96.1	1.26
6	2	3	3	103.0	1.18
1	1	3	3	117.0	1.31
8	2	3	3	97.8	1.52
2	1	3	3	114.0	1.74
9	2	3	3	105.0	1.83
6	2	4	3	83.0	1.31
2	1	4	3	89.0	1.81
9	2	4	3	82.1	1.50
8	2	4	3	73.8	1.22
1	1	4	3	88.1	1.48
11	2	1	3	97.9	1.18
5	1	1	3	108.0	1.34
10	2	1	3	88.8	1.23
3	1	1	3	112.0	1.21
4	1	1	3	117.0	1.52
7	2	1	3	98.7	1.18
4	1	2	3	113.0	1.44
3	1	2	3	113.0	1.46
10	2	2	3	90.0	1.48
5	1	2	3	111.0	1.37
11	2	2	3	94.1	1.34
7	2	2	3	113.0	1.36
3	1	3	3	116.0	1.84
4	1	3	3	116.0	1.77
10	2	3	3	87.8	1.72
11	2	3	3	91.5	1.77
5	1	3	3	111.0	1.71
7	2	3	3	104.0	1.28
7	2	4	3	106.0	1.52
10	2	4	3	90.8	1.70
4	1	4	3	113.0	2.01
11	2	4	3	95.0	1.49
3	1	4	3	110.0	2.06
5	1	4	3	110.0	2.13

ND= NO DATA

TABLE B3: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 100 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
5	1	1	2	124.0	1.27
3	1	1	2	127.0	1.21
11	2	1	2	106.0	1.03
4	1	1	2	120.0	1.16
7	2	1	2	117.0	1.09
10	2	1	2	115.0	1.10
5	1	2	2	127.0	1.49
11	2	2	2	112.0	1.66
7	2	2	2	125.0	1.25
10	2	2	2	113.0	ND
4	1	2	2	130.0	1.26
3	1	2	2	131.0	ND
7	2	3	2	133.0	1.73
10	2	3	2	123.0	2.19
4	1	3	2	141.0	1.80
11	2	3	2	112.0	ND
5	1	3	2	138.0	1.68
3	1	3	2	140.0	1.86
4	1	4	2	119.0	2.32
3	1	4	2	119.0	1.74
7	2	4	2	110.0	1.54
10	2	4	2	102.0	3.85
5	1	4	2	114.0	3.06
11	2	4	2	94.3	1.05
1	1	1	2	87.6	1.36
9	2	1	2	99.2	1.15
2	1	1	2	106.0	1.27
8	2	1	2	91.1	1.19
6	2	1	2	100.0	1.08
6	2	2	2	90.0	1.30
8	2	2	2	67.0	1.05
9	2	2	2	106.0	1.17
2	1	2	2	95.4	1.18
1	1	2	2	95.2	1.36
8	2	3	2	85.7	1.28
1	1	3	2	98.4	1.17
9	2	3	2	85.4	1.38
6	2	3	2	90.6	1.58
2	1	3	2	97.4	1.23
9	2	4	2	83.0	2.87
2	1	4	2	93.6	5.75
1	1	4	2	95.0	1.31
8	2	4	2	84.3	1.79
6	2	4	2	86.1	1.26

ND= NO DATA

TABLE B4: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

CHAMBER #3 TEST CONCENTRATION 100 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)
1	1	1	1	94.6
4	1	1	1	105.0
9	2	1	1	94.3
2	1	1	1	98.4
5	1	1	1	99.6
3	1	1	1	102.0
10	2	1	1	93.6
11	2	1	1	85.1
8	2	1	1	76.6
6	2	1	1	76.7
7	2	1	1	95.7
5	1	2	1	110.0
11	2	2	1	90.9
10	2	2	1	99.8
3	1	2	1	115.0
1	1	2	1	ND
8	2	2	1	80.4
6	2	2	1	86.2
7	2	2	1	106.0
9	2	2	1	97.4
4	1	2	1	114.0
2	1	2	1	104.0
8	2	3	1	71.8
2	1	3	1	90.4
11	2	3	1	85.5
5	1	3	1	161.0
7	2	3	1	97.5
4	1	3	1	114.0
1	1	3	1	86.8
9	2	3	1	86.9
3	1	3	1	114.0
10	2	3	1	99.7
6	2	3	1	75.3
2	1	4	1	113.0
4	1	4	1	111.0
9	2	4	1	109.0
8	2	4	1	91.3
10	2	4	1	97.2
3	1	4	1	111.0
6	2	4	1	90.9
5	1	4	1	109.0
11	2	4	1	82.6
1	1	4	1	109.0
7	2	4	1	118.0

PARTICLE SIZE NOT TAKEN

ND= NO DATA

TABLE B5: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 60 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
5	1	1	1	59.6	ND
11	2	1	1	43.3	ND
7	2	1	1	61.6	ND
4	1	1	1	61.8	ND
3	1	1	1	60.7	ND
10	2	1	1	50.9	ND
10	2	2	1	55.8	ND
4	1	2	1	59.3	ND
7	2	2	1	48.4	ND
11	2	2	1	61.2	ND
5	1	2	1	61.8	ND
3	1	2	1	60.9	ND
4	1	3	1	57.4	ND
7	2	3	1	62.1	ND
11	2	3	1	48.3	ND
5	1	3	1	61.1	ND
10	2	3	1	55.2	ND
3	1	3	1	58.1	ND
7	2	4	1	74.0	ND
4	1	4	1	78.9	ND
3	1	4	1	74.2	ND
11	2	4	1	57.2	ND
10	2	4	1	64.0	ND
5	1	4	1	74.5	ND
8	2	1	1	57.0	1.39
2	1	1	1	66.2	1.34
9	2	1	1	61.3	1.25
1	1	1	1	55.2	1.48
6	2	1	1	58.8	1.28
9	2	2	1	57.0	1.16
1	1	2	1	56.8	1.25
2	1	2	1	60.6	1.43
6	2	2	1	57.5	1.78
8	2	2	1	54.3	1.21
2	1	3	1	54.9	1.53
1	1	3	1	54.4	1.73
6	2	3	1	49.6	1.47
9	2	3	1	50.6	1.26
8	2	3	1	48.8	1.48
8	2	4	1	55.0	1.29
9	2	4	1	57.0	4.66
1	1	4	1	59.9	1.48
2	1	4	1	61.4	1.90
6	2	4	1	56.3	1.40

ND= NO DATA

TABLE B6: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

CHAMBER #5 TEST CONCENTRATION 10 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
5	1	1	1	12.5	1.25
3	1	1	1	12.5	1.64
10	2	1	1	13.1	0.97
4	1	1	1	13.4	3.21
7	2	1	1	14.9	0.94
11	2	1	1	13.4	1.03
5	1	2	1	9.4	1.30
11	2	2	1	7.2	1.33
7	2	2	1	9.5	1.10
3	1	2	1	8.3	1.30
10	2	2	1	9.1	1.76
4	1	2	1	9.0	1.46
4	1	3	1	8.3	1.52
5	1	3	1	7.2	1.37
3	1	3	1	7.7	1.36
10	2	3	1	8.8	0.81
11	2	3	1	8.5	0.65
7	2	3	1	7.8	0.37
10	2	4	1	12.1	0.40
3	1	4	1	11.4	1.55
7	2	4	1	11.0	0.42
11	2	4	1	11.4	0.80
4	1	4	1	12.0	0.83
5	1	4	1	12.0	0.99

ND= NO DATA

TABLE B7: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

CHAMBER #1 TEST CONCENTRATION 60 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
9	2	1	1	62.4	1.11
6	2	1	1	58.4	1.49
11	2	1	1	52.0	1.39
5	1	1	1	48.0	1.72
1	1	1	1	72.4	1.16
3	1	1	1	ND	1.40
10	2	1	1	49.2	1.74
4	1	1	1	57.3	1.98
7	2	1	1	64.5	1.36
8	2	1	1	59.6	1.51
2	1	1	1	70.3	1.12
8	2	2	1	60.5	1.46
9	2	2	1	60.4	1.38
5	1	2	1	49.6	2.52
3	1	2	1	53.4	2.00
7	2	2	1	44.2	1.81
11	2	2	1	42.9	1.78
2	1	2	1	67.4	1.22
4	1	2	1	53.6	2.21
1	1	2	1	63.2	1.97
6	2	2	1	53.5	1.26
10	2	2	1	45.0	1.94
1	1	3	1	61.6	1.18
7	2	3	1	60.7	1.29
10	2	3	1	56.9	2.51
5	1	3	1	65.9	2.10
6	2	3	1	51.3	2.10
11	2	3	1	60.2	1.58
8	2	3	1	49.6	1.50
9	2	3	1	56.1	1.48
4	1	3	1	66.2	3.72
3	1	3	1	69.1	1.67
2	1	3	1	68.6	1.55
10	2	4	1	61.3	ND
6	2	4	1	54.9	1.52
11	2	4	1	55.0	2.69
2	1	4	1	67.6	1.92
8	2	4	1	59.6	2.45
5	1	4	1	64.4	1.92
9	2	4	1	59.2	2.12
7	2	4	1	59.2	3.01
4	1	4	1	64.4	2.47
3	1	4	1	65.3	2.35
1	1	4	1	65.9	2.16

ND= NO DATA

TABLE B8: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

CHAMBER #2 TEST CONCENTRATION 100 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
5	1	1	1	71.9	1.31
7	2	1	1	91.9	1.01
10	2	1	1	78.6	1.04
3	1	1	1	94.5	1.35
4	1	1	1	99.7	1.27
11	2	1	1	85.2	1.14
10	2	2	1	77.3	1.56
3	1	2	1	90.9	1.70
11	2	2	1	79.2	1.11
5	1	2	1	89.1	1.32
7	2	2	1	85.0	1.54
4	1	2	1	88.3	1.90
11	2	3	1	34.2	2.16
7	2	3	1	91.8	0.60
5	1	3	1	106.0	1.68
4	1	3	1	101.0	1.37
10	2	3	1	77.7	1.68
3	1	3	1	100.0	2.19
4	1	4	1	105.0	2.27
3	1	4	1	95.0	1.94
11	2	4	1	92.8	1.89
5	1	4	1	101.0	1.70
7	2	4	1	93.4	1.62
10	2	4	1	83.1	1.49
1	1	1	1	108.0	1.37
8	2	1	1	101.0	1.19
9	2	1	1	85.3	1.07
6	2	1	1	94.1	1.18
2	1	1	1	96.6	1.31
2	1	2	1	95.4	1.13
9	2	2	1	85.2	1.20
6	2	2	1	90.6	1.19
8	2	2	1	88.8	1.09
1	1	2	1	98.9	1.26
1	1	3	1	96.5	2.53
6	2	3	1	87.0	1.66
8	2	3	1	80.6	2.85
2	1	3	1	96.6	1.20
9	2	3	1	85.7	1.43
6	2	4	1	107.0	1.56
9	2	4	1	97.6	1.48
2	1	4	1	111.0	1.52
1	1	4	1	111.0	1.42
8	2	4	1	96.9	1.61

ND= NO DATA

TABLE B9: SOLID PARTICULATE AEROSOL HOMOGENITY STUDY

CHAMBER #5 TEST CONCENTRATION 10 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
8	2	1	1	7.4	1.26
9	2	1	1	8.2	1.40
1	1	1	1	13.4	1.66
2	1	1	1	15.8	1.15
6	2	1	1	11.5	1.60
6	2	2	1	9.2	1.33
1	1	2	1	10.1	1.28
9	2	2	1	8.9	1.26
2	1	2	1	11.3	1.10
8	2	2	1	5.7	1.31
2	1	3	1	8.7	1.46
6	2	3	1	7.0	1.28
1	1	3	1	10.4	1.26
9	2	3	1	7.8	0.99
8	2	3	1	6.7	1.39
6	2	4	1	8.4	1.32
1	1	4	1	10.0	1.95
9	2	4	1	8.5	1.25
2	1	4	1	9.2	1.61
8	2	4	1	6.2	1.18
4	1	1	1	9.7	1.49
3	1	1	1	9.1	1.16
7	2	1	1	10.4	1.48
10	2	1	1	7.7	1.57
5	1	1	1	9.3	1.48
11	2	1	1	8.4	1.56
5	1	2	1	11.5	1.88
7	2	2	1	10.7	1.50
11	2	2	1	10.5	1.22
4	1	2	1	11.5	1.83
3	1	2	1	11.8	1.41
10	2	2	1	10.1	1.32
3	1	3	1	9.8	ND
4	1	3	1	7.4	1.82
10	2	3	1	8.6	0.58
7	2	3	1	9.4	1.63
11	2	3	1	9.0	1.03
5	1	3	1	8.5	2.28
10	2	4	1	7.9	1.31
7	2	4	1	9.5	2.18
4	1	4	1	11.5	1.71
3	1	4	1	9.7	1.89
5	1	4	1	10.3	2.11
11	2	4	1	9.8	1.04

ND= NO DATA

TABLE B10: POSITIVE CONTROL
SOLID PARTICULATE AEROSOL HOMOGENITY STUDY
CHAMBER #4 TEST CONCENTRATION 200mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)
3	1	1	1	173.0
4	1	1	1	189.0
5	1	1	1	193.0
7	2	1	1	164.0
8	2	1	1	140.0
10	2	1	1	140.0
1	1	1	1	241.0
2	1	1	1	235.0
6	2	1	1	181.0
11	2	1	1	164.0
9	2	1	1	164.0
3	1	2	1	204.0
4	1	2	1	223.0
5	1	2	1	224.0
7	2	2	1	189.0
8	2	2	1	164.0
10	2	2	1	161.0
1	1	2	1	245.0
2	1	2	1	231.0
6	2	2	1	185.0
11	2	2	1	163.0
9	2	2	1	170.0
3	1	3	1	202.0
4	1	3	1	217.0
5	1	3	1	216.0
7	2	3	1	173.0
8	2	3	1	160.0
10	2	3	1	154.0
1	1	3	1	219.0
2	1	3	1	210.0
6	2	3	1	164.0
11	2	3	1	146.0
9	2	3	1	154.0
3	1	4	1	166.0
4	1	4	1	178.0
5	1	4	1	177.0
7	2	4	1	152.0
8	2	4	1	129.0
10	2	4	1	124.0
1	1	4	1	205.0
2	1	4	1	195.0
6	2	4	1	149.0
11	2	4	1	131.0
9	2	4	1	134.0

PARTICLE SIZE REPORTED SEPERATLY

TABLE B11: SPATIAL VARIATION OF PARTICLE SIZE[a]

CHAMBER 4 POSITIVE CONTROL

PORT	PARTICLE SIZE(microns)
1	2.95
2	3.28
3	3.05
4	3.07
5	3.11
6	3.07
7	2.75
8	3.09
9	3.04
10	2.54

[a] MEASURED BY MERCER CASCADE IMPACTOR

TABLE B12: TEMPORAL VARIATION OF PARTICLE SIZE[a]

CHAMBER 4 POSITIVE CONTROL

TIME HOURS	PARTICLE SIZE(microns)
1	3.1
2	3.4
3	3.04
4	2.98

[a] MEASURED BY MERCER CASCADE IMPACTOR

TABLE B 13: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 1200 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	1250.0	0.198
2	1	1	1	1090.0	0.432
3	1	1	1	775.0	0.469
4	1	1	1	1090.0	0.253
5	1	1	1	1100.0	0.244
6	2	1	1	1230.0	0.273
7	2	1	1	1090.0	0.499
8	2	1	1	1090.0	0.276
9	2	1	1	1070.0	0.279
10	2	1	1	994.0	0.423
11	2	1	1	1080.0	0.276
1	1	2	1	1230.0	0.520
2	1	2	1	1280.0	0.324
3	1	2	1	1250.0	0.479
4	1	2	1	1150.0	0.358
5	1	2	1	1220.0	0.419
6	2	2	1	1120.0	0.378
7	2	2	1	1110.0	0.358
8	2	2	1	1230.0	0.551
9	2	2	1	1106.0	0.629
10	2	2	1	1080.0	0.324
11	2	2	1	1200.0	0.551
1	1	3	1	1450.0	0.308
2	1	3	1	1400.0	0.311
3	1	3	1	1150.0	0.428
4	1	3	1	1150.0	0.289
5	1	3	1	1110.0	0.280
6	2	3	1	1450.0	0.332
7	2	3	1	1250.0	0.345
8	2	3	1	1130.0	0.459
9	2	3	1	1280.0	0.593
10	2	3	1	1030.0	0.517
11	2	3	1	1090.0	0.459
1	1	4	1	1310.0	0.339
2	1	4	1	1230.0	0.294
3	1	4	1	1260.0	0.312
4	1	4	1	1260.0	0.488
5	1	4	1	1240.0	0.407
6	2	4	1	1230.0	0.579
7	2	4	1	1330.0	0.415
8	2	4	1	1270.0	0.553
9	2	4	1	1160.0	0.439
10	2	4	1	1090.0	0.581
11	2	4	1	1260.0	0.553

TABLE B14: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 1000 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	1040.0	0.295
2	1	1	1	1030.0	0.270
3	1	1	1	1060.0	0.312
4	1	1	1	1050.0	0.297
5	1	1	1	1040.0	0.338
6	2	1	1	1040.0	0.374
7	2	1	1	1040.0	0.344
8	2	1	1	960.0	0.316
9	2	1	1	1040.0	0.443
10	2	1	1	1000.0	0.386
11	2	1	1	961.0	0.316
1	1	2	1	1040.0	0.252
2	1	2	1	1090.0	0.236
3	1	2	1	1030.0	0.339
4	1	2	1	1050.0	0.267
5	1	2	1	1050.0	0.315
6	2	2	1	1030.0	0.479
7	2	2	1	1030.0	0.430
8	2	2	1	980.0	0.437
9	2	2	1	1040.0	0.471
10	2	2	1	1010.0	0.407
11	2	2	1	981.0	0.437
1	1	3	1	1050.0	0.277
2	1	3	1	1090.0	0.225
3	1	3	1	1040.0	0.274
4	1	3	1	999.0	0.247
5	1	3	1	1050.0	0.263
6	2	3	1	1000.0	0.450
7	2	3	1	1030.0	0.404
8	2	3	1	975.0	0.522
9	2	3	1	1040.0	0.381
10	2	3	1	970.0	0.449
11	2	3	1	980.0	0.522
1	1	4	1	1070.0	0.245
2	1	4	1	1100.0	0.257
3	1	4	1	1040.0	0.315
4	1	4	1	1010.0	0.367
5	1	4	1	1040.0	0.327
6	2	4	1	1030.0	0.261
7	2	4	1	1010.0	0.287
8	2	4	1	970.0	0.322
9	2	4	1	1070.0	0.326
10	2	4	1	1010.0	0.370
11	2	4	1	936.0	0.322

TABLE B15: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 700 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	2	1	1	755.0	0.291
2	1	1	1	719.0	0.244
3	1	1	1	687.0	0.192
4	1	1	1	685.0	0.194
5	1	1	1	692.0	0.305
6	2	1	1	697.0	0.293
7	2	1	1	695.0	0.312
8	2	1	1	660.0	1.005
9	2	1	1	665.0	0.467
10	2	1	1	624.0	0.388
11	2	1	1	643.0	1.005
1	1	2	1	742.0	0.247
2	1	2	1	740.0	0.219
3	1	2	1	703.0	0.248
4	1	2	1	694.0	0.475
5	1	2	1	676.0	0.572
6	2	2	1	659.0	0.374
7	2	2	1	615.0	0.299
8	2	2	1	695.0	0.271
9	2	2	1	676.0	0.319
10	2	2	1	603.0	0.361
11	2	2	1	626.0	0.271
1	1	3	1	745.0	0.280
2	1	3	1	727.0	0.228
3	1	3	1	687.0	0.270
4	1	3	1	670.0	0.243
5	1	3	1	698.0	0.308
6	2	3	1	726.0	0.358
7	2	3	1	689.0	0.344
8	2	3	1	637.0	0.324
9	2	3	1	726.0	0.410
10	2	3	1	557.0	0.349
11	2	3	1	605.0	0.324

No data was collected for the fourth hour.

TABLE B16: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 700 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC.(mg/m^3)	PARTICLE SIZE(microns)
1	1	1	2	609.0	0.256
2	1	1	2	615.0	0.434
3	1	1	2	740.0	0.491
4	1	1	2	717.0	0.235
5	1	1	2	702.0	0.358
6	2	1	2	701.0	0.762
7	2	1	2	774.0	0.439
8	2	1	2	690.0	0.802
9	2	1	2	723.0	0.475
10	2	1	2	643.0	0.448
11	2	1	2	694.0	0.802
1	1	2	2	753.0	0.280
2	1	2	2	722.0	0.242
3	1	2	2	719.0	0.270
4	1	2	2	740.0	0.281
5	1	2	2	734.0	0.497
6	2	2	2	680.0	0.399
7	2	2	2	729.0	0.544
8	2	2	2	632.0	0.412
9	2	2	2	685.0	0.333
10	2	2	2	599.0	0.427
11	2	2	2	665.0	0.412
1	1	3	2	746.0	0.282
2	1	3	2	755.0	0.251
3	1	3	2	739.0	0.367
4	1	3	2	720.0	0.583
5	1	3	2	738.0	0.403
6	2	3	2	697.0	0.407
7	2	3	2	708.0	0.376
8	2	3	2	630.0	0.508
9	2	3	2	689.0	0.524
10	2	3	2	682.0	0.370
11	2	3	2	632.0	0.508
1	1	4	2	730.0	0.326
2	1	4	2	736.0	0.262
3	1	4	2	712.0	0.281
4	1	4	2	725.0	0.302
5	1	4	2	763.0	0.312
6	2	4	2	667.0	0.295
7	2	4	2	703.0	0.384
8	2	4	2	646.0	0.464
9	2	4	2	666.0	0.475
10	2	4	2	618.0	0.566
11	2	4	2	679.0	0.464

TABLE B17: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

PILOT CHAMBER (CHAMBER #3) TEST CONCENTRATION 700 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	3	759.0	0.417
2	1	1	3	754.0	0.368
3	1	1	3	733.0	0.256
4	1	1	3	778.0	0.246
5	1	1	3	713.0	0.290
6	2	1	3	695.0	0.286
7	2	1	3	767.0	4.356
8	2	1	3	675.0	0.312
9	2	1	3	712.0	0.310
10	2	1	3	629.0	0.300
11	2	1	3	673.0	0.312
1	1	2	3	760.0	0.244
2	1	2	3	798.0	0.481
3	1	2	3	801.0	0.274
4	1	2	3	813.0	0.254
5	1	2	3	801.0	0.372
6	2	2	3	714.0	0.347
7	2	2	3	872.0	0.354
8	2	2	3	768.0	0.414
9	2	2	3	733.0	0.437
10	2	2	3	654.0	0.411
11	2	2	3	714.0	0.414
1	1	3	3	698.0	0.921
2	1	3	3	738.0	0.207
3	1	3	3	762.0	0.269
4	1	3	3	740.0	0.300
5	1	3	3	746.0	0.332
6	2	3	3	693.0	0.354
7	2	3	3	876.0	0.401
8	2	3	3	777.0	0.421
9	2	3	3	701.0	0.405
10	2	3	3	633.0	0.493
11	2	3	3	739.0	0.421
1	1	4	3	759.0	0.241
2	1	4	3	740.0	0.228
3	1	4	3	744.0	0.187
4	1	4	3	735.0	0.253
5	1	4	3	708.0	0.503
6	2	4	3	731.0	0.364
7	2	4	3	788.0	0.455
8	2	4	3	764.0	0.414
9	2	4	3	720.0	0.383
10	2	4	3	615.0	0.585
11	2	4	3	700.0	0.414

TABLE B18: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

CHAMBER #1 TEST CONCENTRATION 700 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	683.0	0.240
2	1	1	1	699.0	0.181
3	1	1	1	700.0	0.417
4	1	1	1	733.0	0.292
5	1	1	1	787.0	0.237
6	2	1	1	700.0	0.275
7	2	1	1	703.0	0.339
8	2	1	1	637.0	0.284
9	2	1	1	678.0	0.294
10	2	1	1	696.0	0.424
11	2	1	1	654.0	0.284
1	1	2	1	708.0	0.464
2	1	2	1	688.0	0.518
3	1	2	1	685.0	0.201
4	1	2	1	682.0	0.171
5	1	2	1	690.0	0.237
6	2	2	1	681.0	0.291
7	2	2	1	670.0	0.291
8	2	2	1	626.0	0.316
9	2	2	1	667.0	0.321
10	2	2	1	650.0	0.349
11	2	2	1	642.0	0.316
1	1	3	1	697.0	0.246
2	1	3	1	689.0	0.172
3	1	3	1	683.0	0.198
4	1	3	1	694.0	0.192
5	1	3	1	696.0	0.216
6	2	3	1	700.0	0.257
7	2	3	1	671.0	0.537
8	2	3	1	626.0	0.348
9	2	3	1	691.0	0.317
10	2	3	1	647.0	0.355
11	2	3	1	617.0	0.348
1	1	4	1	729.0	0.186
2	1	4	1	767.0	0.155
3	1	4	1	692.0	0.199
4	1	4	1	693.0	0.170
5	1	4	1	685.0	0.178
6	2	4	1	749.0	0.393
7	2	4	1	679.0	0.468
8	2	4	1	669.0	0.363
9	2	4	1	681.0	0.263
10	2	4	1	675.0	0.333
11	2	4	1	643.0	0.363

TABLE B19: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

CHAMBER #2 TEST CONCENTRATION 700 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	721.0	0.348
2	1	1	1	738.0	0.264
3	1	1	1	710.0	0.213
4	1	1	1	731.0	0.297
5	1	1	1	817.0	0.249
6	2	1	1	732.0	0.311
7	2	1	1	674.0	0.329
8	2	1	1	674.0	0.373
9	2	1	1	713.0	0.444
10	2	1	1	663.0	0.370
11	2	1	1	654.0	0.373
1	1	2	1	748.0	0.332
2	1	2	1	756.0	0.265
3	1	2	1	752.0	0.287
4	1	2	1	803.0	0.231
5	1	2	1	822.0	0.354
6	2	2	1	781.0	0.354
7	2	2	1	709.0	0.479
8	2	2	1	671.0	0.386
9	2	2	1	712.0	0.448
10	2	2	1	707.0	0.369
11	2	2	1	692.0	0.386
1	1	3	1	732.0	0.274
2	1	3	1	728.0	0.194
3	1	3	1	714.0	0.266
4	1	3	1	751.0	0.255
5	1	3	1	821.0	0.398
6	2	3	1	770.0	0.306
7	2	3	1	694.0	0.351
8	2	3	1	679.0	0.339
9	2	3	1	704.0	0.807
10	2	3	1	702.0	0.295
11	2	3	1	678.0	0.339
1	1	4	1	825.0	0.256
2	1	4	1	808.0	0.211
3	1	4	1	748.0	0.386
4	1	4	1	754.0	0.542
5	1	4	1	819.0	0.352
6	2	4	1	880.0	0.299
7	2	4	1	712.0	0.311
8	2	4	1	715.0	0.369
9	2	4	1	745.0	0.355
10	2	4	1	692.0	0.442
11	2	4	1	680.0	0.369

TABLE B20: SOLID/LIQUID MIXTURE AEROSOL HOMOGENITY STUDY

CHAMBER #5 TEST CONCENTRATION 1200 mg/m^3

PORT	SHELF	TIME	REPLICATE	AEROSOL CONC. (mg/m^3)	PARTICLE SIZE (microns)
1	1	1	1	1133.0	0.393
2	1	1	1	1236.0	0.423
3	1	1	1	1105.0	0.405
4	1	1	1	1066.0	0.305
5	1	1	1	1136.0	0.305
6	2	1	1	1160.0	0.286
7	2	1	1	1058.0	0.347
8	2	1	1	1069.0	0.352
9	2	1	1	1049.0	4.409
10	2	1	1	1080.0	0.366
11	2	1	1	1146.0	0.352
1	1	2	1	1199.0	0.304
2	1	2	1	1184.0	0.289
3	1	2	1	1157.0	0.280
4	1	2	1	1200.0	0.401
5	1	2	1	1188.0	0.389
6	2	2	1	1191.0	0.396
7	2	2	1	1118.0	0.361
8	2	2	1	1120.0	0.391
9	2	2	1	1140.0	0.418
10	2	2	1	1126.0	0.438
11	2	2	1	1170.0	0.391
1	1	3	1	1240.0	0.272
2	1	3	1	1244.0	0.399
3	1	3	1	1151.0	0.347
4	1	3	1	1139.0	0.369
5	1	3	1	1177.0	0.362
6	2	3	1	1154.0	0.490
7	2	3	1	1090.0	0.496
8	2	3	1	1129.0	0.489
9	2	3	1	1180.0	0.425
10	2	3	1	1102.0	0.297
11	2	3	1	1146.0	0.489
1	1	4	1	1259.0	0.365
2	1	4	1	1230.0	0.329
3	1	4	1	1189.0	0.327
4	1	4	1	1351.0	0.341
5	1	4	1	1288.0	0.350
6	2	4	1	1171.0	0.399
7	2	4	1	1218.0	0.494
8	2	4	1	1137.0	0.456
9	2	4	1	1291.0	0.589
10	2	4	1	1234.0	0.584
11	2	4	1	1172.0	0.456

APPENDIX C

STATISTICAL REPORT

CONTRACT # DAMD17-89-C-9043

INHALATION TOXICITY OF SINGLE MATERIALS AND MIXTURES

Statistical Report on the Chamber Atmosphere Homogeneity Studies

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January 24, 1990

1 Introduction

The purpose of this report is to describe the statistical methods and results for the evaluation of the chamber atmosphere homogeneity studies. Three sets of data were analyzed: the solid particulate data, the solid liquid mixture data, and the positive control data. For each, the parameters under study were actual concentration and particle size, which were analyzed statistically using a components of variance analysis of variance model (Winer, 1971, *Statistical principles in experimental design, 2nd edition*, McGraw-Hill). Prior to analysis, the actual values were log transformed to better accomodate the assumed normality of the statistical model.

The primary purpose of these analyses was to estimate the amount of spatial and temporal variability within the chambers and to determine whether these estimated variances represented significant variation about the estimated mean value of the chamber. For this determination, a criterion of 20% of the estimated mean value was used; if the variance estimate accounted for 20% or more of the variation about the estimated mean it was deemed to be significant. Finally, inter-chamber analyses were performed, for those chambers exposed to the same target concentration, to investigate whether significant variation was attributable to the chamber and to examine the homogeneity of the estimated spatial and temporal variation across chambers.

2 Solid Particulate Distribution Study

Data from the target concentrations of 10, 60, 100, and 200 mg/m^3 were obtained within the pilot chamber (chamber # 3), while data from chambers 5, 1, and 2 corresponded to the target concentrations of 10, 60 and 100 mg/m^3, respectively. Within each chamber, actual concentration and particle size data were collected from both top and bottom shelves of the chamber and at four hourly timepoints following exposure of the target concentration. Sample ports 1-5 were considered top shelf locations and ports 6-10 were bottom shelf locations. Additionally, since all ports could not be assessed at the same time, the ports were divided into two sets: set one consisting of sample ports 3,4,5,7,8, and 10, while set two consisted of sample ports 1,2,6,8, and 9. Sample port 8 was included in both sets to assess and adjust for any inter-set differences. For the target concentration of 100 mg/m^3 in the pilot chamber, data were collected at three replications.

2.1 Intra-chamber Analysis

Within each chamber (and for the pilot chamber, within each target concentration) three sources of variability were of interest: the variation due to shelf (σ^2_α), time (σ^2_β), and set (σ^2_γ). The variation due to shelf and time represent, respectively, the spatial and temporal variability in the chamber. In terms of interactions, the variability due to shelf *by* time ($\sigma^2_{\alpha\beta}$) was also estimated, in order to test the homogeneity of shelf variance across time. The expected mean squares for this three way components of

variance model are given as:

source	symbol	expected mean square
shelf	α	$\sigma_\epsilon^2 + nr\sigma_{\alpha\beta}^2 + nqr\sigma_\alpha^2$
time	β	$\sigma_\epsilon^2 + nr\sigma_{\alpha\beta}^2 + npr\sigma_\beta^2$
set	γ	$\sigma_\epsilon^2 + npq\sigma_\gamma^2$
shelf x time	$\alpha\beta$	$\sigma_\epsilon^2 + nr\sigma_{\alpha\beta}^2$
residual	ϵ	σ_ϵ^2

with $p = 2$ shelf levels, $q = 4$ timepoints, and $r = 2$ levels of the set factor. Also, n refers to the cell sample size in each of the 16 cells formed by this 4 x 2 x 2 design; since sample port 8 was assessed twice the design is not completely balanced, and so, the average cell sample size of 2.75 can be used for n. For the target concentration of 100 mg/m^3 in the pilot chamber, the variability due to replication (σ_δ^2) must also be accounted for, and so, the components of variance for this target concentration are given as:

source	symbol	expected mean square
shelf	α	$\sigma_\epsilon^2 + nrs\sigma_{\alpha\beta}^2 + nqrs\sigma_\alpha^2$
time	β	$\sigma_\epsilon^2 + nrs\sigma_{\alpha\beta}^2 + nprs\sigma_\beta^2$
set	γ	$\sigma_\epsilon^2 + npqs\sigma_\gamma^2$
replication	δ	$\sigma_\epsilon^2 + npqr\sigma_\delta^2$
shelf x time	$\alpha\beta$	$\sigma_\epsilon^2 + nrs\sigma_{\alpha\beta}^2$
residual	ϵ	σ_ϵ^2

where $s = 3$ for the three replications.

The results from these variance component analyses are given in Tables 1 (for actual concentration) and 2 (for particle size). In all cases the shelf x time interaction was non-significant, and so was not included in the final tables. As can be seen from Table 1, the coefficient of variation for actual concentration never exceeds 20% in any of the chambers at any target concentration. Additionally, the means for the actual concentrations are usually very close to the target concentrations and always within one standard devation of the target value.

For particle size, given in Table 2, many of the coefficients of variation exceed 20%, however, the percentage of variation due to the shelf or time effects never exceeds 20%. Furthermore, the actual values for the mean and standard deviations in these chambers are quite small and within an acceptable range. It should be noted that for the target concentration of 100 mg/m^3 in the pilot chamber, while for the actual concentration data there were 3 replications, for particle size only two replications were obtained and so the sample size listed differs for the particle size

($n = 85$) and actual concentration ($n = 131$) data. Also, particle size data for the target concentration of 60 mg/m^3 in the pilot chamber were obtained only for the second set of sample ports (1,2,6,8, and 9) and thus the sample size listed in Table 2 is only 20 for this target concentration within the pilot chamber.

2.2 Inter-chamber Analysis

For each target concentration, where data from more than one chamber were available (10, 60, and 100 mg/m^3), an analysis was performed to assess the chamber variability and the homogeneity of the time and shelf effects across chambers. Four sources of variability were of interest: the variation due to time, shelf, set, and chamber (σ^2_ς). In terms of interactions, the variability due to chamber by shelf ($\sigma^2_{\alpha\varsigma}$) and chamber by time ($\sigma^2_{\beta\varsigma}$) were estimated in order to test the homogeneity of temporal and spatial variability across chambers. The expected mean squares for this four way components of variance model are given as:

source	symbol	expected mean square
shelf	α	$\sigma^2_\epsilon + nqr\sigma^2_{\alpha\varsigma} + nqrt\sigma^2_\alpha$
time	β	$\sigma^2_\epsilon + npr\sigma^2_{\beta\varsigma} + nprt\sigma^2_\beta$
set	γ	$\sigma^2_\epsilon + npqt\sigma^2_\gamma$
chamber	ς	$\sigma^2_\epsilon + nqr\sigma^2_{\alpha\varsigma} + npr\sigma^2_{\beta\varsigma} + npqr\sigma^2_\varsigma$
chamber x shelf	$\alpha\varsigma$	$\sigma^2_\epsilon + nqr\sigma^2_{\alpha\varsigma}$
chamber x time	$\beta\varsigma$	$\sigma^2_\epsilon + npr\sigma^2_{\beta\varsigma}$
residual	ϵ	σ^2_ϵ

where t indicates the number of chambers in the analysis.

The results from these variance component analyses are given in Tables 3 (for actual concentration) and 4 (for particle size). The coefficient of variation for actual concentration, listed in Table 3, does not exceed 20% for any of the three target concentrations. Furthermore, the variability due to the interactions of chamber x time and chamber x shelf is small, indicating that the low degree of spatial and temporal variability is consistent across chambers. The overall means for the actual concentrations are very close to the target concentrations and always within one standard devation of the target value. In the analysis of the 100 mg/m^3 data, only the first replication was used so that the actual number of pilot and non-pilot chamber data points were comparable.

For particle size, given in Table 4, all of the coefficients of variation exceed 20%, however, the percentage of variation due to any of the model effects never exceeds 20%. Again, the actual values for the mean and standard deviations in these chambers are quite small and within an acceptable range. In the analysis of the 100 mg/m^3 particle size data, only one of the replications was used so that the actual number of pilot and non-pilot chamber data points were comparable. For the

same reason, in the analysis of the particle size data for the target concentration of 60 mg/m^3, only the data from the second set of sample ports were included.

3 Solid Liquid Mixture Distribution Study

Data from the target concentrations of 700, 1000, and 1200 were obtained within the pilot chamber (chamber # 3), while data from chambers 1 and 2 corresponded to the target concentration of 700, and data from chamber 5 corresponded to the target concentrations of 1200. Within each chamber, actual concentration and particle size data were collected from both top and bottom shelves of the chamber and at four hourly timepoints following exposure of the target concentration. Sample ports 1-5 were considered top shelf locations and ports 6-10 were bottom shelf locations. The ports were again divided into two sets, corresponding to their actual time of assessment: set one consisting of sample ports 3,4,5,7,8, and 10, while set two consisted of sample ports 1,2,6,8, and 9. Sample port 8 was included in both sets to assess and adjust for any inter-set differences only for the actual concentration determinations; for the particle size determinations only one sample was obtained from sample port 8. For the target concentration of 700 in the pilot chamber, data were collected at three replications, although in the first replication only 3 hours of data were obtained.

3.1 Intra-chamber Analysis

Within each chamber (and for the pilot chamber, within each target concentration) three sources of variability were of interest: the variation due to time, shelf, and set. In terms of interactions, the variability due to time by shelf was also estimated, in order to test the homogeneity of shelf variance across time. The expected mean squares for this three way components of variance model is given in section 2.1. For the target concentration of 700 in the pilot chamber, the replication factor is also included and the components of variance for this model are also given in section 2.1.

The results from these variance component analyses are given in Tables 5 (for actual concentration) and 6 (for particle size). In all cases the shelf x time interaction was non-significant, and so was not included in the final tables. As can be seen from Table 5, the coefficient of variation for actual concentration is typically small and never exceeds 20% in any of the chambers at any target concentration. The means for the actual concentrations are usually very close to the target concentrations and always within one standard devation of the target value.

For particle size, given in Table 6, all of the coefficients of variation exceed 20%, however, the percentage of variation due to the time effect never exceeds even 5%, while the percentage of variation attributable to the shelf effect exceeds the 20% cutpoint only in chamber 1. Furthermore, the actual values for the mean and standard deviations in these chambers are quite small and within an acceptable range.

3.2 Inter-chamber Analysis

For each target concentration, where data from more than one chamber were available (700 and 1200), an analysis was performed to assess the homogeneity of the time and shelf effects across chambers. Four sources of variability were of interest: the variation due to time, shelf, set, and chamber. In terms of interactions, the variability due to chamber by time and chamber by shelf were estimated in order to test the homogeneity of temporal and spatial variability across chambers. The expected mean squares for this four way components of variance model is given as in section 2.2.

The results from these variance component analyses are given in Tables 7 (for actual concentration) and 8 (for particle size). The coefficient of variation for actual concentration, listed in Table 7, does not exceed 20% for either of the two target concentrations. Furthermore, the variability due to the interactions of chamber x time and chamber x shelf is very small, indicating that the low degree of spatial and temporal variability is consistent across chambers. The overall means for the actual concentrations are very close to the target concentrations and always within one standard devation of the target value. In the analysis of the 700 data, only one of the replications within the pilot chamber was used so that the actual number of pilot and non-pilot chamber data points were comparable.

For particle size, given in Table 8, both of the coefficients of variation exceed 20%, however, the percentage of variation due to any of the model effects never exceeds 20%. In light of the finding that the shelf effect of chamber 1 exceeded 20%, it is important to note that the overall variability due to shelf at the concentration of 700 is below 20%. Also, the actual values for the mean and standard deviations in these chambers are quite small and within an acceptable range. In the analysis of the 700 particle size data, only one of the replications was used so that the actual number of pilot and non-pilot chamber data points were comparable.

4 Positive Control Material Distribution Study

Data from the target concentration of 200 mg/m^3 were obtained within chamber # 4. The actual concentration was collected from both top and bottom shelves of the chamber and at four hourly timepoints following exposure of the target concentration. Sample ports 1-5 were considered top shelf locations and ports 6-10 were bottom shelf locations. The ports were again divided into two sets, corresponding to their actual time of assessment: set one consisting of sample ports 3,4,5,7,8, and 10, while set two consisted of sample ports 1,2,6,8, and 9. Sample port 8 was included in both sets to assess and adjust for any inter-set differences.

Within the chamber three sources of variability were of interest: the variation due to time, shelf, and set. In terms of interactions, the variability due to time by shelf was also estimated, in order to test the homogeneity of shelf variance across time. The expected mean squares for this three way components of variance model is given in section 2.1.

The results of the variance component analysis of actual concentration are given in Table 9. From the table, we see that the sample size is $n = 44$ and the mean of 179.55 with a standard deviation of 32.45 yields a coefficient of variation of 18.07%. Since the shelf x time interaction was non-significant, it was not included in the final model. The coefficient of variation for actual concentration does not exceed 20% and the mean for the actual concentrations is easily within one standard devation of the target value.

For particle size, it was difficult to obtain sufficient data to perform the components of variance model. Instead, in order to estimate a coefficient of variation for the temporal effect, one sample per hour for four hours was obtained in chamber 4 at sample port 5. For these data the mean of 3.13 with a standard deviation of 0.19 yields a coefficient of variation of roughly 6%. Similarly, to estimate a coefficient of variation for the spatial effect, one sample per port was obtained in chamber 5. Here, the mean of 3.00 with a standard devation of 0.21 yields a coefficient of variation of approximately 7%. Thus, from these limited data, we can conclude that no appreciable effects due to shelf or time were observed for particle size.

5 Summary

In the analysis of the actual concentration data, the estimated coefficients of variation never exceeded 20% and the estimated means were always within a standard deviation of the target value. This was observed in both the intra- and inter-chamber analyses. For the particle size data, while the estimated coefficients of variation always exceeded 20%, only once did the variability due to shelf or time exceed 20%: in the analysis of the liquid solid mixture data, in one chamber (# 1) the shelf variation was estimated to be 24% of the mean value. However, in the inter-chamber analysis the shelf effect was not above 20%, and the estimated means and standard devations were always very small and within an acceptable range for particle size data. Thus, these results provide strong support for the homogeneity of the chamber atmosphere for the three sets of data analyzed: the solid particulate data, the solid liquid mixture data, and the positive control data.

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Study Statistician
January 24, 1990

TABLE 1

Components of Variance for the Solid Particulate
Aerosol Concentrations in the Exposure Chambers

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 10 mg/m^3								
44	10.60	1.83	17.3%		0.8%	0.2%	13.1%	3.3%
pilot chamber - target concentration = 60 mg/m^3								
44	58.70	7.12	12.1%		0.6%	3.2%	3.2%	5.1%
pilot chamber - target concentration = 100 mg/m^3								
131	103.01	15.85	15.4%	0.9%	5.5%	4.9%	0.2%	3.9%
pilot chamber - target concentration = 200 mg/m^3								
44	197.25	17.95	9.1%		2.5%	3.9%	0.2%	2.5%
chamber 5 - target concentration = 10 mg/m^3								
44	9.47	1.87	19.8%		0.3%	8.1%	2.3%	9.1%
chamber 1 - target concentration = 60 mg/m^3								
43	58.86	7.38	12.5%		2.3%	5.2%	2.4%	2.6%
chamber 2 - target concentration = 100 mg/m^3								
44	92.65	9.45	10.2%		1.6%	3.8%	1.5%	3.4%

TABLE 2

Components of Variance for the Solid Aerosol
Particle Size in the Exposure Chambers

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 10 mg/m^3								
44	1.13	0.48	42.5%		0.0%	12.5%	15.1%	14.9%
pilot chamber - target concentration = 60 mg/m^3								
20	1.59	0.75	47.2%			0.0%	2.1%	45.1%
pilot chamber - target concentration = 100 mg/m^3								
85	1.55	0.65	41.8%	0.0%	1.1%	2.8%	14.7%	23.2%
pilot chamber - target concentration = 200 mg/m^3								
44	1.96	0.51	26.0%		1.6%	0.3%	12.5%	11.6%
chamber 5 - target concentration = 10 mg/m^3								
43	1.45	0.34	23.4%		0.8%	5.4%	0.3%	16.9%
chamber 1 - target concentration = 60 mg/m^3								
43	1.86	0.55	29.7%		10.4%	0.0%	8.4%	10.9%
chamber 2 - target concentration = 100 mg/m^3								
44	1.50	0.43	28.7%		0.0%	1.1%	6.5%	21.1%

TABLE 3

Components of Variance for the Solid Particulate
Aerosol Concentrations between various Exposure Chambers

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 10 mg/m^3										
88	10.03	1.93	19.2%	0.0%	1.5%	1.8%	0.2%	5.6%	3.4%	6.7%
target concentration = 60 mg/m^3										
87	58.78	7.21	12.3%	0.0%	4.8%	1.0%	0.0%	2.1%	0.0%	4.3%
target concentration = 100 mg/m^3										
87*	95.71	13.18	13.8%	0.0%	5.8%	0.8%	0.9%	0.4%	0.7%	5.2%

* *only the first replication data within the pilot chamber were included*

TABLE 4

Components of Variance for the Solid Aerosol
Particle Size between various Exposure Chambers

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 10 mg/m^3										
87	1.29	0.45	34.5%	0.0%	6.6%	0.0%	5.9%	9.2%	0.9%	11.9%
target concentration = 60 mg/m^3										
40[1]	1.58	0.59	37.3%		0.0%	9.7%	0.0%	0.0%	0.0%	27.6%
target concentration = 100 mg/m^3										
85[2]	1.56	0.69	44.2%	0.0%	1.1%	13.0%	0.0%	0.4%	0.0%	29.6%

[1] *only the second set data within the non-pilot chamber were included*
[2] *only the second replication data within the pilot chamber were included*

TABLE 5

Components of Variance for the Solid Liquid Mixture
Aerosol Concentrations in the Exposure Chambers

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 700								
121	706.51	54.46	7.7%	1.7%	0.0%	2.0%	0.0%	4.0%
pilot chamber - target concentration = 1000								
44	1025.1	36.95	3.6%		0.3%	1.6%	0.0%	1.7%
pilot chamber - target concentration = 1200								
44	1181.0	122.6	10.4%		2.2%	0.2%	2.7%	5.4%
chamber 1 - target concentration = 700								
44	684.82	34.17	5.0%		0.1%	2.1%	0.4%	2.4%
chamber 2 - target concentration = 700								
44	734.82	52.35	7.1%		0.2%	3.1%	0.6%	3.2%
chamber 5 - target concentration = 1200								
44	1166.4	65.51	5.6%		0.2%	1.2%	2.2%	2.0%

TABLE 6

Components of Variance for the Solid Liquid Mixture
Aerosol Particle Size in the Exposure Chambers

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
pilot chamber - target concentration = 700								
110	0.37	0.14	37.8%	2.3%	0.0%	12.2%	0.0%	23.2%
pilot chamber - target concentration = 1000								
40	0.35	0.09	25.7%		0.0%	13.9%	1.0%	10.8%
pilot chamber - target concentration = 1200								
40	0.40	0.11	27.5%		0.0%	5.6%	3.8%	18.2%
chamber 1 - target concentration = 700								
40	0.29	0.10	34.5%		0.0%	23.8%	1.1%	9.6%
chamber 2 - target concentration = 700								
40	0.34	0.11	32.4%		0.0%	12.1%	0.0%	20.3%
chamber 5 - target concentration = 1200								
40	0.39	0.08	20.5%		0.0%	8.0%	1.1%	11.4%

TABLE 7

Components of Variance for the Solid Liquid Mixture
Aerosol Concentrations between various Exposure Chambers

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 700										
132*	705.39	49.13	7.0%	0.0%	2.4%	0.0%	1.5%	0.3%	0.0%	2.8%
target concentration = 1200										
88	1173.7	98.00	8.3%	1.1%	0.6%	2.2%	0.0%	0.2%	0.0%	4.2%

* *only the second replication data within the pilot chamber were included*

TABLE 8

Components of Variance for the Solid Liquid Mixture
Aerosol Particle Size between various Exposure Chambers

				Variance Components						
n	mean	standard deviation	coefficient of variation	set	shelf	time	chamber	chamber x time	chamber x shelf	residual
target concentration = 700										
120*	0.35	0.12	34.3%	0.2%	14.5%	0.0%	6.8%	0.7%	0.0%	12.1%
target concentration = 1200										
80	0.39	0.10	25.6%	0.0%	6.9%	2.0%	0.0%	0.8%	0.0%	15.8%

* *only the second replication data within the pilot chamber were included*

TABLE 9

Components of Variance for the Aerosol Concentrations
of Positive Control Material in the Exposure Chambers

		standard	coefficient		*Variance Components*			
n	mean	deviation	of variation	rep	set	shelf	time	residual
chamber 4 - target concentration = 200 mg/m^3								
44	179.55	32.45	18.1%		0.6%	13.2%	2.6%	1.7%

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